Microbiomics | Sample Collection & Preservation Kits

PRODUCT GUIDE 2025

Life Science Research

DANAGEN

Index

Microbiomics

Sample Collection & Preservation Kits

What is a microbiome?

The NIH Human Microbiome Project defines the microbiome as the collective genomes of the microbes (composed of bacteria, bacteriophage, fungi, protozoa and viruses) that reside inside living beings. The human microbiota consists of the 10-100 trillion symbiotic microbial cells, primarily contained within the gut, but also in the mouth, the skin, the nose, the vagina, etc. The human microbiome consists of the genes these cells harbour.

Various terminologies can create confusion: for example, "microbiota" (the microbial taxa associated with humans) and "microbiome" (the catalogue of these microbes and their genes) are often interchanged. In addition, "metagenomics" originally referred to shotgun characterization of total DNA, although now it is increasingly being applied to studies of marker genes such as the 16S rRNA gene."

Why is it so important?

Bacterial Cells > Human Cells: We contain about 10 times as many microbial cells as human cells. The human gut microbiota consists of many different types of bacteria - over 1000 bacterial species have been identified. However, the presence and prevalence of these bacteria changes person to person, with only approximately 160 species per person per fecal sample. This variance is based on many factors: bacterial infections, antibiotic treatment, lifestyle, surgeries, dietary changes or other health changes.

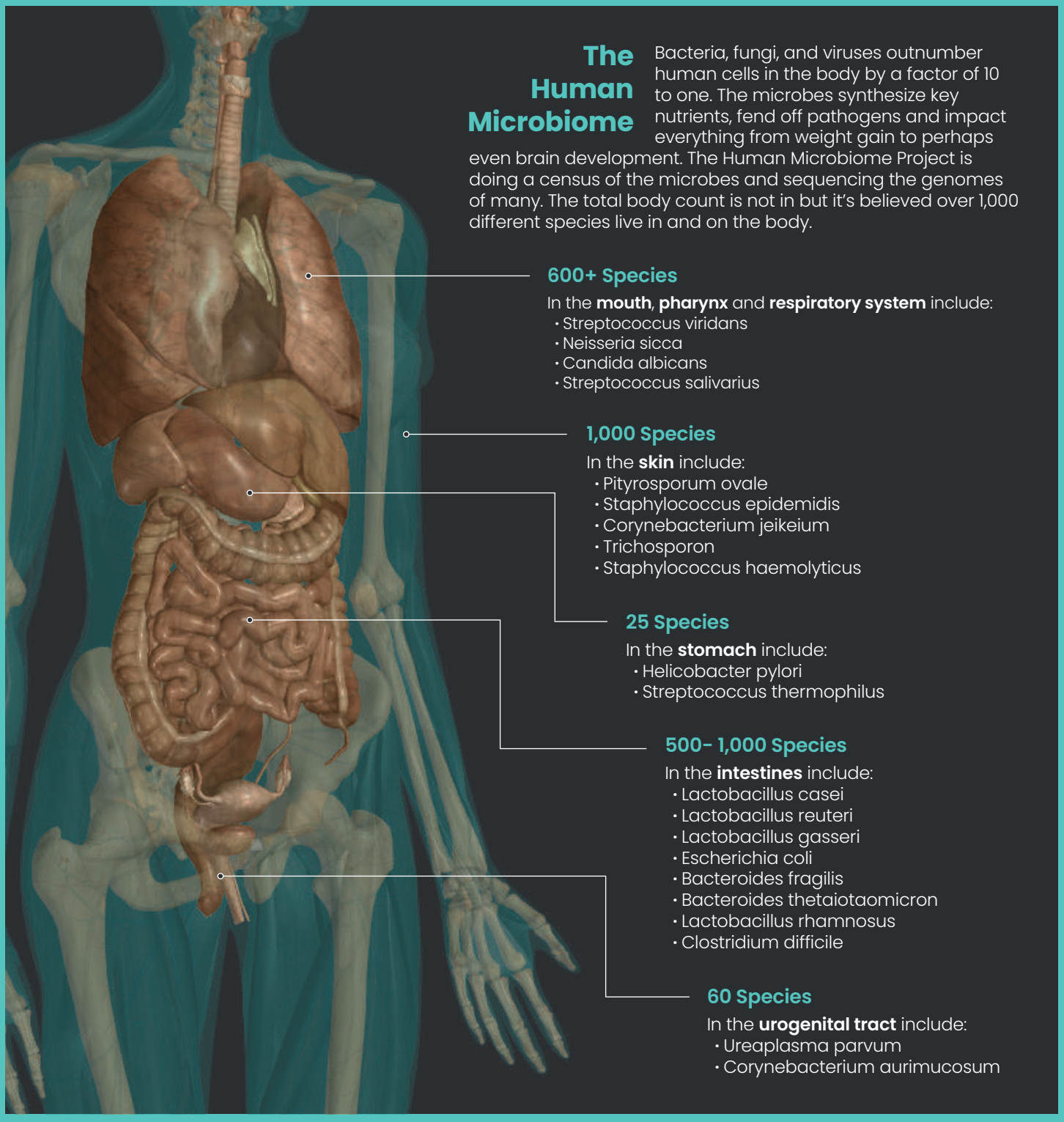
Impact on Health: An analysis of the full gene content and composition of the microbiomes living in the human body (i.e. the metagenome) predicts that there may be more than 8 million unique microbial genes associated with the microbiomes across the human body of healthy adults. When compared to the total number of human genes, this suggests that the genetic contribution of the microbiome to the human supraorganism may be many hundreds of times greater than the genetic contribution from the human genome. This means that the human gut microbiome can have a profound effect on the types of diseases we encounter and how we heal/recover.

The importance
of sample collection & stabilization

Maintaining the integrity of samples collected is a major challenge in microbiome research. From the point of collection, the microbial composition of a sample can begin to change.

Active microbial samples can alter their composition easily in response to changes in the environment. Therefore, all microbial samples require preservation methods if subsequent processing does not happen immediately. For this purpose, most researchers have been relying on freezing or refrigeration, which unfortunately is too inconvenient or costly to implement in many circumstances, e.g. collecting and transporting thousands of samples from individual homes and in the wild. This challenge leads to a need for convenient cold- free methods for microbial sample collection, preservation, and transportation.

Bacteria, fungi, and viruses outnumber human cells in the body by factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perphas even brain development. The human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it's believed over 1,000 different species live in and on the body.



Choose **DANAGEN** for your microbiome studies.

Sample collection/stabilization and DNA/RNA extraction from swab samples for microbiome or metagenome analyses

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Introduction

Good preservation and storage are essential to preserving microorganisms' genetic material in microbial communities from wide array of sample inputs and accurately represent the bacterial composition for further analysis and applications.

The objective is to develop a proper preservation and storage medium to preserve DNA and RNA from those microorganisms.

DANAGEN-BIOTED has developed a new product to deal with this problem:

A. DANASWAB Sample Collection MICROBIOME Kit: is designed for the collection, ambient storage and transport from samples collected using a swab wherever a swab may be deemed appropriate per application.

Material and Methods

· Sample Collection

200 mg of human stool samples were collected using DANASWAB Sample Collection MICROBIOME Kit and stored at room temperature for 1 month.

· Microbial DNA/RNA isolation

Preserved stool samples were obtained at the indicated time points (Figure 1) and processed following the DANAGENE MICROBIOME SWAB DNA kit protocol for DNA isolation and the DANAGENE Microbial RNA kit for RNA isolation (Figure 2).

· PCR amplification of 16S rRNA genes and targeted library preparations and sequencing

Genomic DNA amplification was conducted out in duplicate, using the 16S 1-24 Barcode Kit (SQK-16S024; Oxford Nanopore Technologies, Oxford, UK) with the following PCR conditions:

Initial denaturation at 95°C for 5 minutes, 25 cycles of 95°C for 20s, 55°C for 30s, and 65°C for 2 minutes, followed by a final extension at 65°C for 5 minutes (Kai et al., 2019).

Amplifies were purified using CleanNGS (CleanNA, PH Waddinxveen, The Netherlands) and quantified by fluorometric quantification with Qubit (Thermo Fisher Scientific).

A total of 333ng of DNA was used for library preparation and sequenced in MiniON flow cells (FLO-FLG001; Oxford Nanopore Technologies) according to the manufacturer's protocol. After 24 hours of sequencing, the total number of reads for each sample ranged from 35,000 to 43,000.

Results

The microbiome composition and abundance of stool samples conserved 0 and 30 days in DANASWAB Sample Collection MICROBIOME Kit designed to preserve DNA and RNA do not present significant differences. The composition of both samples is constant (Figure 1 and Table 1). Microbial RNA isolated with the DANAGENE Microbial RNA kit presents an optimal quality. Quality was assessed using the Agilent 4150 TapeStation System (Figure 2), showing clearly RNA fragments corresponding to 23S, 16S, microRNAs, and an absence of degradation.

	Day 0	Day 30
Species	238	238
Individuals	22978	26714
Simpson-D	0.9753	0.9725
Shannon-H	4,315	4,269
Sorensen	100%	

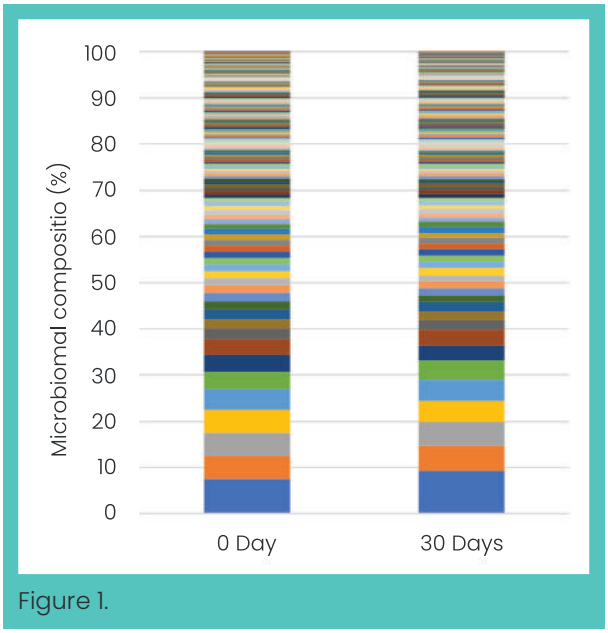

Figure 1.

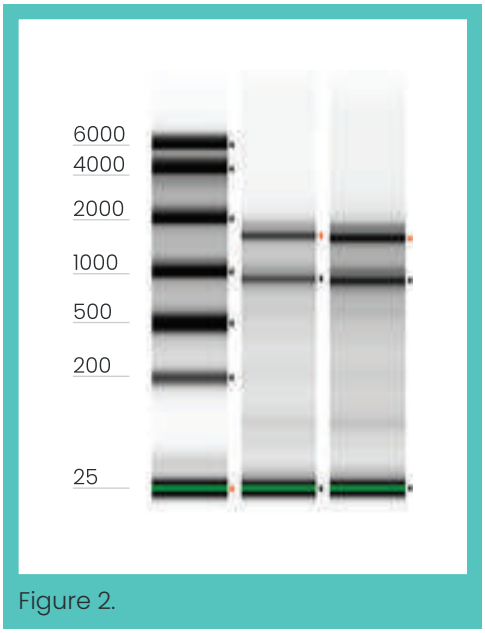

Figure 2.

Conclusions

According to the Shannon and Simpson index, it can be determined that both samples show high biodiversity, and a qualitative similarity of 100% between them.

The MICROBIOME Stabilization Solution takes a microbial snapshot and preserves RNA of a sample while inactivating pathogens making samples safe and ready for transport. Samples stored in the these microtubes are stable at ambient temperature for 1 month, and can be frozen for longer-term storage.

Reference

Kai S, Matsuo Y, Nakagawa S, Kryukov K, Matsukawa S, Tanaka H, Iwai T, Imanishi T & Hirota K. Rapid bacterial identification by direct PCR amplification of 16S rRNA genes using the MinION™ nanopore sequencer. FEBS Open Bio 9(3), 548–557 (2019). https://doi.org/10.1002/2211-5463.12590

For more information, visit *www.danagen.es/en*

DANAGEN

BioTechniques
The International Journal of Life Science Methods

Sample Collection / Preservation and DNA Isolation from stool samples for MICROBIOME analysis

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Introduction

Appropriate preservation and storage of stool samples is crucial to maintain DNA integrity and microbial community composition for downstream applications and analysis, including NGS and microbiome characterization.

The ultimate goal of a microbiome analysis is to reveal the real composition of a microbial community. To achieve an accurate representation of the original sample, collection/storage and isolation methods need to prevent the alteration of the nucleic acids profile to avoid inaccuracies and biases.

DANAGEN-BIOTED has developed two products to overcome these challenging tasks:

A. DANASTOOL Sample Collection MICROBIOME Kit enables collection, storage and stabilization of stool samples. It comes in a tube with a spoon and liquid preservative solution that preserves the microbiome composition.

B. DANAGENE MICROBIOME Fecal DNA Kit designed for fast and easy purification of DNA from preserved stool samples using an optimized lysis method.

Material and Methods

· Sample Collection

0.5–1.0 gr of human stool samples were collected using DANASTOOL Sample Collection MICROBIOME Kit and stored at room temperature for two months.

· Microbial DNA Isolation

Preserved stool samples were obtained at the indicated time points (Figure 1) and processed following the DANAGENE MICROBIOME Fecal DNA kit protocol.

To determine if our DNA extraction method is biased or not, we have used a mock microbial community containing known quantities of different microbes.

· Targeted Library Preparations, Sequencing and Bioinformatics Analysis

The samples were processed and analysed with the ZymoBIOMICS Service:Targeted Metagenomic Sequencing (Zymo Research, Irvine,CA).

Results

Microbial composition of stool samples preserved at room temperature is unchanged after 15 days and with minimum changes after two months stored in DANASTOOL preservative solution. Samples had a constant microbial composition (Figure 1).

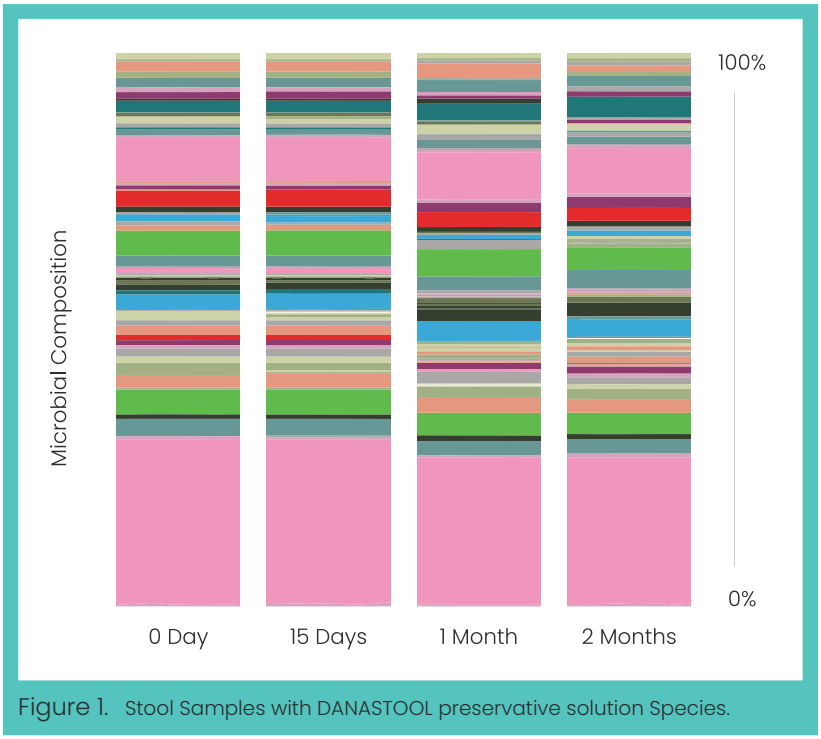


Figure 1. Stool Samples with DANASTOOL preservative solution Species.

Conclusions

The DANASTOOL preservative solution preserves microbiota profiles for unbiased and reproducible results and eliminates sample variability.

Furthermore, the preservative solution and our DNA isolation method maintain DNA integrity.

In this paper we demonstrate that DANASTOOL Sample Collection MICROBIOME Kit preserves DNA profile of microbial samples stored at room temperature for one or two months, making it ideal for the transportation of stool samples for MICRIOBIOME Analysis.

Validation of DANAGENE Microbiome DNA Kits for Microbiome Analysis

DANAGENE Microbiome DNA Extraction kits

Introduction

Bias in nucleic acid extraction procedures is a major contributor to inaccurate microbial profiling due to inferior cell lysis methods failing to extract DNA uniformly from diverse microbes.

There are several reports in the literature citing variations in microbial composition profiling caused by the use of different DNA extraction methods.

With identification and abundance being the most important factors in a microbiome analysis, lysis efficiency and bioburden/background contamination should be major considerations when using a DNA isolation system. Problems with these two factors can completely distort the truth.

DANAGENE Microbiome DNA kits were built specifically for microbiome analysis and was designed with these new requirements in mind. To determine if a microbial DNA extraction process is biased or not, one needs a microbial sample of defined composition.

Unbiased cellular lysis was validated using one Microbial Community Standard.

Material and Methods

· Microbial Community Standard

We prepared one microbial community standard with the following composition of Table 1:

Species	Theorical Abum. (%)
Veillonella rogosae	48.50
Faecalibacterium prausnitzii	22.50
Eschericiha coli	7.50
Lactobacillus fermentum	6.50
Roseburia hominis	6.50
Clostridium difficile	2.50
Prevotella corporis	2.00
Bacteroides fragilis	1.50
Fusobacterium nucleatum	1.50
Salmonella enterica	0.50
Akkermansia muciniphilia	0.50

· DNA Extraction from Microbial Community Standard

75 µl of Standard was used to compare different DNA extractions protocols:

A. DANAGENE Microbiome Fecal DNA Kit.

B. DANAGENE Microbiome Swab DNA Kit.

C. Supplier Z.

D. Supplier Q.

· Targeted Library Preparations, Sequencing and Bioinformatics Analysis

Genomic DNA amplification was conducted out in duplicate, using the 16S 1-24 Barcode Kit (SQK-16S024; Oxford Nanopore Technologies, Oxford, UK) with the following PCR conditions:
Initial denaturation at 95 ° C for 5 minutes, 32 cycles of 95 ° C for 30s, 53 ° C for 45s, and 65°C for 2 minutes and 15s, followed by a final extension at 65 ° C for 5 minutes.
Amplifies were purified using CleanNGS (CleanNA, PH Waddinxveen, The Netherlands) and quantified by fluorometric quantification with Qubit (Thermo Fisher Scientific).
A total of 333ng of DNA was used for library preparation and sequenced in MiniON flow cells (FLO-FLG001; Oxford Nanopore Technologies) according to the manufacturer's protocol. After 24 hours of sequencing, the total number of reads for each sample ranged from 35,000 to 43,000.

Results

We can observe a little variation in microbial composition compared with the standard composition using our DANAGENE Microbiome Extraction kits for fecal and soil samples. Despite these kits used different chemistry and beads for mechanical lysis. (Fig.1)

In addition, we also have good results with the species in low percentages and better results that Supplier Z and Q.

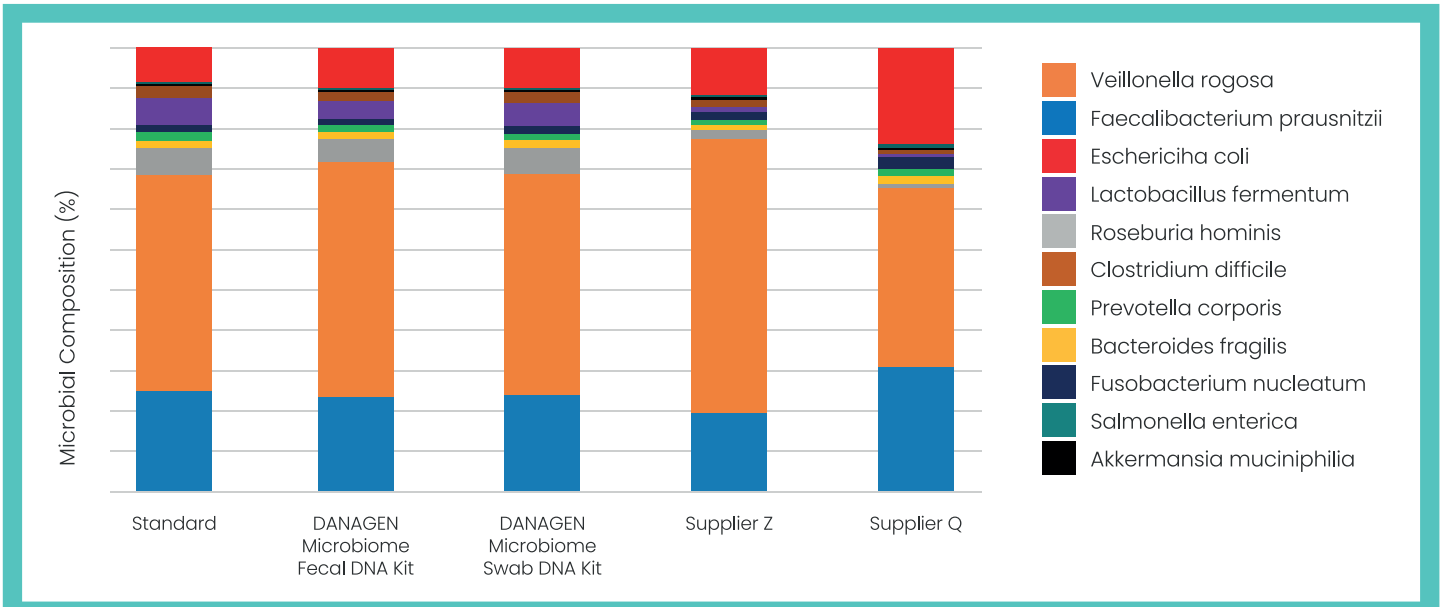


Figure 1.

Conclusion

The goal of this study was the validation of our DANAGENE Microbiome DNA extraction kits for microbiome analysis due the DNA extraction can be biased because of uneven microbial cell lysis or low bioburden. For this, we prepared one Microbial community standard for comparing different DNA extractions protocols.

In conclusion, our data demonstrate that our isolation kits for stool and soil samples can be used for an efficient DNA isolation for microbiome analysis.

Metagenomic analysis of the human vaginal microbiome with a vaginal self-collection swab & Microbiome Vaginal Kit

DANAGENE Microbiome Vaginal DNA kit

Introduction

The **vaginal microbiome** is a specific compartment of the human microbiome. Unique conditions of the vagina are characterized by a few microbial species, usually lactobacilli.

The cervicovaginal ecosystem is made up of diverse microorganisms coexisting in a dynamic balance and establishing complex connections with each other and with the host. In healthy reproductive-aged women, the vaginal microbiome, generally, shows a predominance of Lactobacillus genus, and most women display the prevalence of one species among *L. crispatus, L. iners, L. jensenii* and *L. gasseri*. Lactobacilli promote the maintenance of the vaginal homeostasis and prevent the colonization and growth of adverse microorganisms, including those responsible for sexually transmitted infections (STI). The composition of the vaginal microbiome depends on age, menstruations, hormonal fluctuations, sexual behaviors, and also the use of drugs such as probiotics and antibiotics causing its imbalance.

Material and Methods

· Sample Collection

Vaginal samples were taken using our **Vaginal Self-Collection Swab** (Danagen) a new solution for home self-sampling, collection, shipping and easy processing in the laboratory from 4 women, they were not pregnant, of reproductive age, ranging from 20 to 45 years and regularly menstruating.

· Whole-Genomic DNA Extraction from Vaginal Swabs

Swab samples were stored for 1 week to room temperature, then preserved vaginal samples were processed following the **DANAGENE MICROBIOME Vaginal DNA kit** protocol (Danagen).

· Targeted Library Preparations, Sequencing and Bioinformatics Analysis

The extracted DNA was quantified using Quant-IT PicoGreen (Invitrogen). The sequencing libraries.

Are prepared according to the Illumina 16S Metagenomic Sequencing Library protocols to amplify the V3 and V4 region. The input gDNA 2ng was PCR amplified with 5x reaction buffer, 1mM of dNTP mix, 500nM each of the universal F/R PCR primer, and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The cycle condition for 1st PCR was 3 min at 95°C for heat activation, and 25 cycles of 30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C, followed by a 5-min final extension at 72°C. The universal primer pair with Illumina adapter overhang sequences used for the first amplifications were as follows:

V3-F: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′
V4-R: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′

The 1st PCR product was purified with AMPure beads (Agencourt Bioscience, Beverly, MA). Following purification, the 2ul of 1st PCR product was PCR amplified for final library construction containing the index using NexteraXT Indexed Primer. The cycle condition for 2nd PCR was same as the 1st PCR condition except for 10 cycles. The PCR product was purified with AMPure beads. The final purified product is then quantified using qPCR according to the qPCR Quantification Protocol Guide(KAPA Library Quantificatoin kits for IlluminaSequecing platforms) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). The paired-end (2×300 bp) sequencing was performed by the Macrogen using the MiSeq™ platform (Illumina, San Diego, USA).

Sequencing reads were analysed with the cloud metagenomics software GAIA (*Paytuví et al.*, 2019)

(https://metagenomics.sequentiabiotech.com) to obtain OTU tables at different taxonomic levels with their corresponding Shannon alpha-diversity and Bray-Curtis beta-diversity values.

Results

· Taxonomic composition

The analysis was performed on four samples, two of them showed profile extremely rich in Lactobacilli while the other two samples showed a profile that could be classified as a Community State Type (CST) IV, rich in anaerobic bacteria with a very low abundance of the Lactobacillus genus (figure 1). Results show that the taxonomic composition observed in the samples is consistent with the current knowledge about the expected genera and abundance in the vaginal microbiome.

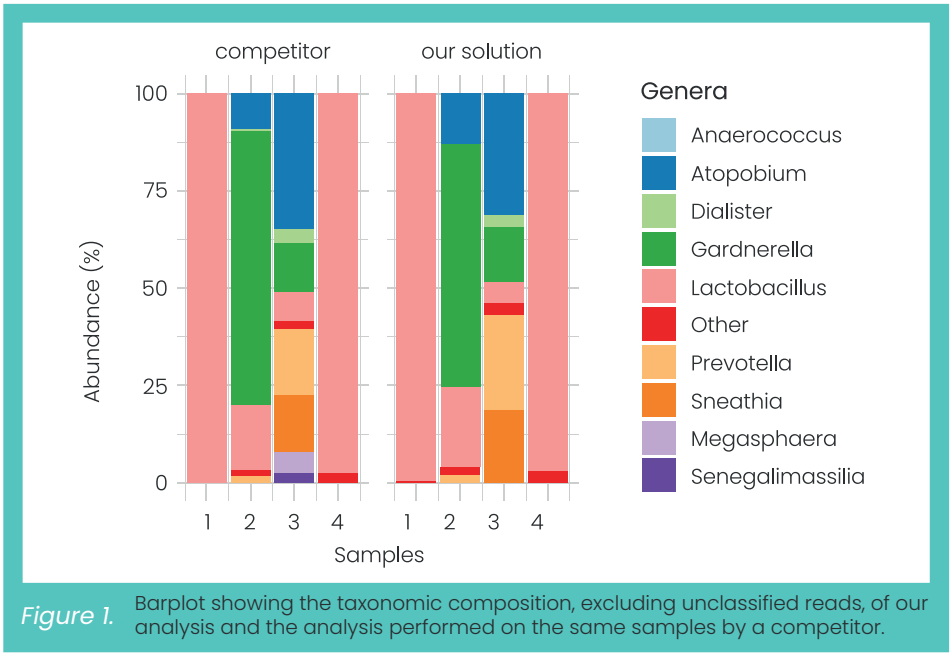


Figure 1. Barplot showing the taxonomic composition, excluding unclassified reads, of our analysis and the analysis performed on the same samples by a competitor.

· Competitors

The same samples were sent to our competitor in order to compare the taxonomic composition of the samples. The taxonomic profile was visually very similar to the one observed with our solution. In terms of Bray-Curtis dissimilarities, the average value between samples was 0.064, suggesting that the taxonomic profile is very similar, thus confirming that our solution is suitable for the analysis of the vaginal microbiome.

Conclusion

When building a house, any good metagenomic analysis is founded in a proper starting material, efficient and reproducible DNA isolation method and bioinformatic analysis to give you the maximum information on your sample.

In this study it has been demonstrated that our system for sampling, DNA isolation and bioinformatic analysis of the vaginal microbiome can be used for an efficient characterization of the vaginal microbiome of asymptomatic and sexually active women.

DANAGEN

SEQUENTIA
DECODE · DISCOVER · DISRUPT

Novel DANAGENE Microbiome Fecal DNA kit for improved results in stool microbiome and metagenomic analysis

DANAGENE Microbiome Fecal DNA Kit

Introduction

Our **New DANAGENE Microbiome Fecal DNA kit** is even more effective than our original Microbiome Fecal technology and is designed to isolate high yields of pure microbial DNA from stool samples for microbiome and metagenomic analysis.

The kit features a novel **Microbial DNA Column** and optimized chemistry for a more efficient removal of PCR inhibitors (such as polysaccharides or bile) using a novel **Microbiome Lysis Buffer and Microbiome Precipitation Buffer**.

In this application note, we are going to study the DNA yield and Microbial composition (%) using the commercially available gold standard for stool samples, the **QIAamp PowerFecal Pro DNA Kit QIAGEN**, versus the **New DANAGENE Microbiome Fecal DNA Kit**.

We also are going to evaluate the use of our new technology for microbial DNA isolation of preserved stool samples in our 2 systems: DANASTOOL Sample Collection Microbiome Kit and DANASWAB Sample Collection Microbiome kit.

Material and Methods

· Stool sample collection

We collected a stool sample from a healthy individual and we preserved it in the following way:

A. Fresh sample without preservation solution.

B. 800 mg stool sample in 8.0 ml DANASTOOL Sample Collection MICROBIOME Kit.

C. 200 mg stool sample in 2.0 ml DANASWAB Sample Collection MICROBIOME Kit.

· DNA isolation

DNA was isolated from stool samples using either the QIAamp PowerFecal Pro DNA Kit, the DANAGENE Microbiome Fecal DNA kit or the DANAGENE Microbiome DANASTOOL Kit protocol. For each protocol, three samples were processed.

Briefly, for the New DANAGENE Microbiome Fecal DNA kit, the microorganisms were efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads. Proteins and Inhibitors were eliminated by precipitation using a new proprietary inhibitor removal buffer and subsequently pelleted by centrifugation together with the beads and undissolved sample material. The purified lysate was mixed with the Binding Buffer and then applied to a new Microbial DNA column. The DNA that is bound to the column underwent a two-wash step. After a drying step, ready-to-use DNA could be eluted with Elution Buffer (5 mM Tris/HCl, pH 8.5).

· Quality and Quantification of extracted DNA

For DNA quantification, DNA concentration was determined fluorometrically on the Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) using the QUBIT dsDNA BR Assay Kit.

For DNA quality, DNA purity was determined via 260/280 and 260/230 ratios measured on the NanoDrop (Thermo Fisher Scientific, USA).

· 16S rRNA Gene Sequencing

Genomic DNA amplification was conducted using the 16S Barcoding Kit (SQK-RAB204; Oxford Nanopore Technologies, Oxford, UK) with the following PCR conditions:
Initial denaturation at 95°C for 1 minute, 25 cycles of 95°C for 20s, 55°C for 30s, and 65°C for 2 minutes, followed by a final extension at 65°C for 5 minutes.
Amplifies were purified using Agencourt AMPure XP beads (Beckman Coulter) and quantified by fluorometric quantification with Qubit (Thermo Fisher Scientific).

A total of 100 ng of DNA was used for library preparation and sequenced in MiniON flow cells (FLO-FLG001; Oxford Nanopore Technologies) according to the manufacturer's protocol. Sequencing reads were analysed with the metagenomics software EPI2ME.

Results and discussion

· Experiment 1

We present a comparison of DNA yield and microbial composition (%) from the same fresh stool sample using the commercially available gold standard QIAamp PowerFecal Pro DNA kit versus our New DANAGENE Microbiome Fecal DNA Kit.

We have obtained similar yield and A260/280 and 260/230 ratios (Table 1). The results are the average of three samples.

The microbial composition resulting from each extraction method (DANAGEN versus QIAGEN) is summarized graphically in Figure 1. Similar results can be observed in terms of microbial composition between our kit and the gold standard for stool samples.

	100 mg Fresh Stool Sample	
KIT	DANAGENE Microbiome Fecal DNA Kit	QIAamp PowerFecal Pro DNA Kit
Yield (ng/µl) QUBIT	164,50	152
A260/280	1,85	1,84
A260/230	1,92	1,94

Table 1. DNA concentrations of total DNA obtained and 260/280 and 260/230 ratios

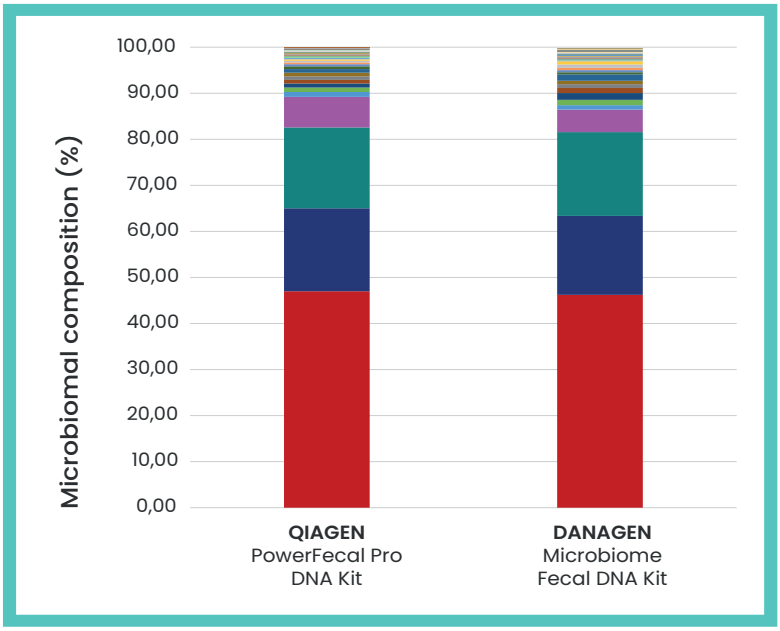


Figure 1. **Microbial composition of stool samples. Results are summarized at the genus level**
DNA was extracted from the same fresh stool sample (100 mg) Kit using the QIAamp PowerFecal Pro DNA kit versus our New DANAGENE Microbiome Fecal DNA Kit. The bacterial composition of each sample type resulting from each extraction protocol is summarized graphically at the genus level.

· Experiment 2

The goal of this experiment was to evaluate whether the new kit is compatible with our fecal sample preservation systems.

We present a comparison of DNA yield and microbial composition (%) using our New DANAGENE Microbiome Fecal DNA Kit from the same stool sample preserved in different ways.

We have obtained similar yield and A260/280 and 260/230 ratios (Table 2). The results are the average of three samples.

We have obtained good results but the best ones are from the fresh stool sample because we can only process 250 µl and 400 µl from DANASTOOL and DANASWAB samples, respectively. The chemistry of both preservative solutions does not allow processing larger samples.
The microbial composition resulting from each extraction protocol is summarized graphically in Figure 2.

Sample	Fresh Stool Sample	Preserved Stool Sample in DANASTOOL	Preserved Stool Sample in DANASWAB
Sample Quantity	100 mg	250 µl	400 µl
Yield (ng/µl) QUBIT	178	40	106
A260/280	1,87	1,84	1,85
A260/230	2,01	1,95	1,82

Table 2. DNA concentrations of total DNA obtained and 260/280 and 260/230 ratios

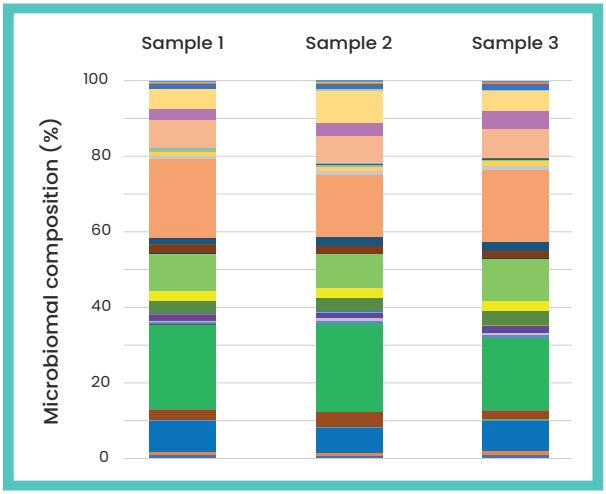


Figure 2. **Microbial composition of stool samples. Results are summarized at the genus level**
DNA was extracted from the same stool sample preserved in different conditions using the New DANAGENE Microbiome Fecal DNA kit. The bacterial composition of each sample type resulting from each extraction protocol is summarized graphically at the genus level. For the preservation of stool samples, we used our 2 solutions.
Sample 1: Fresh Stool sample.
Sample 2: Preserved stool sample in DANASTOOL Sample Collection MICROBIOME Kit.
Sample 3: Preserved stool sample in DANASWAB Sample Collection MICROBIOME Kit.

· Experiment 3

The goal of this experiment was to evaluate the best option for microbial DNA isolation from preserved stool samples in our gold preservation system DANASTOOL Sample MICROBIOME Collection Kit.

We present a comparison of DNA yield and microbial composition (%) using our DANAGENE Microbiome DANASTOOL Kit specifically developed for this kind of samples versus of our New DANAGENE Microbiome Fecal DNA Kit and QIAamp PowerFecal Pro DNA kit.

We have obtained similar A260/280 and 260/230 ratios but total DNA yields were 2.5 times higher for the DANAGENE Microbiome DANASTOOL Kit than for alternative methods (Table 3). The results are the average of three samples.

The microbial composition resulting from each extraction protocol is summarized graphically in Figure 3. Similar results can be observed in terms of microbial composition between the three kits.

Preserved Stool Sample in DANASTOOL Sample MICROBIOME Collection KIt			
Sample	DANAGENE Microbiome DANASTOOL Kit	DANAGENE Microbiome Fecal DNA Kit	QIAamp PowerFecal Pro DNA Kit
Sample Quantity	1.0 ml	250 µl	250 µl
Yield (ng/µl) QUBIT	108	166	160
Elution Volume (µl)	200	50	50
Total Yield (µg)	21,60	8,30	8,0
A260/280	1,90	1,86	1,87
A260/230	2,06	2,07	2,01

Table 3. DNA concentrations of total DNA obtained and 260/280 and 260/230 ratios

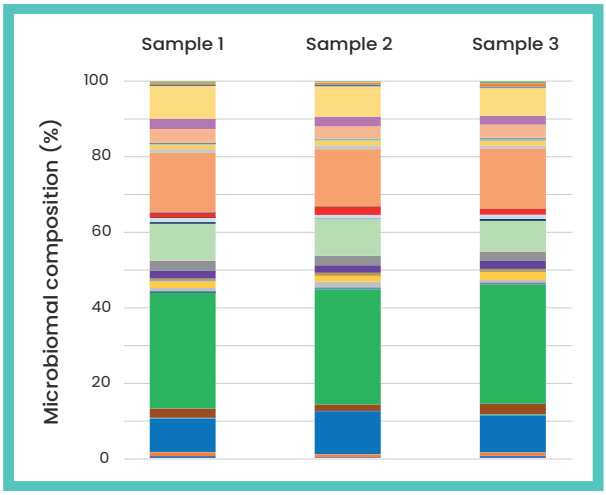


Figure 3. **Microbial composition of stool samples. Results are summarized at the genus level**
DNA was extracted from the same preserved stool sample in DANASTOOL Sample Collection MICROBIOME Kit using different DNA isolation kits. The bacterial composition of each sample type resulting from each extraction protocol is summarized graphically at the genus level.
Sample A: DANAGENE Microbiome DANASTOOL Kit.
Sample B: DANAGENE Microbiome Fecal DNA kit.
Sample 3: QIAamp PowerFecal Pro DNA kit.

Conclusion:

• Designed for rapid purification of highly pure microbial DNA for microbiome analysis.
Our data demonstrate that our new DANAGENE Microbiome Fecal DNA kit has better results than our previous version of DNA isolation kit for fresh stool samples (data not shown) and we obtained similar results in comparison with the gold standard in microbiome stool sample prep, the QIAamp PowerFecal Pro DNA kit (QIAGEN).

• The DANAGENE Microbiome Fecal DNA kit can be used for a fast and efficient microbial DNA isolation from fresh stool samples and preserved stool samples in DANASTOOL and DANASWAB sample Collection MICROBIOME Kit.

• The best option for microbial DNA isolation from fresh stool samples is the New DANAGENE Microbiome Fecal DNA kit.

• The best option for microbial DNA from preserved stool samples in DANASTOOL Sample Collection Microbiome Kit is the old DANAGENE Microbiome DANASTOOL Kit.

Ordering information:

Reference	Product	Preps
0620	DANAGENE Microbiome FECAL DNA Kit	50
0620.50 DANASTOOL	DANAGENE Microbiome DANASTOOL Kit	50
0620.500 DANASTOOL	DANAGENE Microbiome DANASTOOL Kit	250
0617	DANASTOOL Sample Collection MICROBIOME kit	50
0618	DANASTOOL Sample Collection MICROBIOME kit	250
0626.100	DANASWAB Sample Collection MICROBIOME Kit	100

Novel DANAGENE Microbiome Soil DNA kit for improved results in soil microbiome and metagenomic analysis

DANAGENE Microbiome Soil DNA Kit

David Navarro, DANAGEN.BIOTED S.L, Barcelona,Spain, david@danagen.es; Alberto Acedo Bécares, Patricia Alonso Macho, Fernando Moreda Alonso, Felipe Melis, Beatriz Garcia Jimenez, BIOME MAKERS SPAIN S.L., Valladolid, Spain.

Introduction

The **New DANAGENE Microbiome Soil DNA kit** has been designed for the isolation of high molecular weight genomic DNA from microorganisms such as Gram positive and Gram negative bacteria, archaea and fungi **from environmental samples of all soil types**.

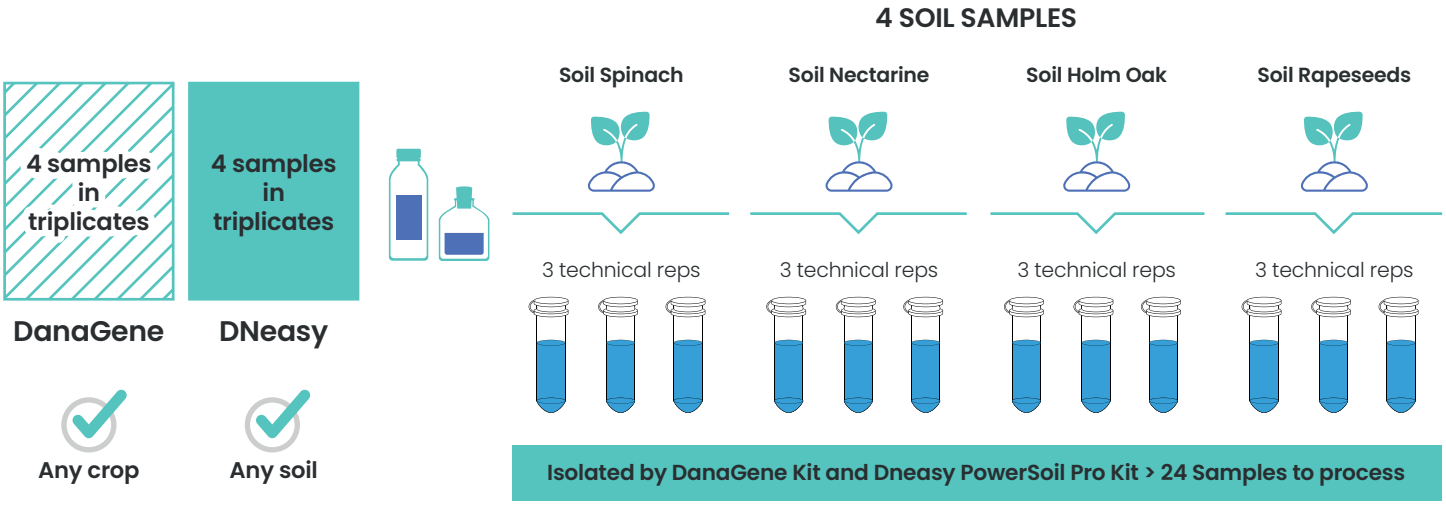
In this application note is compared alfa and beta diversity from four different soil types prepared using the commercially available **DNeasy PowerSoil Pro Kit QIAGEN** versus the **New DANAGENE Microbiome Soil DNA Kit**.

For this, we have used the BeCrop® Test technology (BIOMEMAKERS, Spain), an amplicon sequence analysis of bacteria and fungi applied to soil samples to decipher microbiome identification.

Material and Methods

· Stool sample collection

We collected four soil samples, each one from a different origin (soil spinach, soil nectarine, soil holm oak and soil rapeseeds), three replicates each were analyzed.



· DNA isolation

DNA was isolated from soil samples using either the DNeasy PowerSoil Pro Kit or the DANAGENE Microbiome Soil DNA kit protocol. For each protocol, three 250 mg samples were processed. Briefly, for the DANAGENE Microbiome Soil DNA kit, the microorganisms were efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads. Proteins and Inhibitors were eliminated by precipitation using a new proprietary inhibitor removal buffer and subsequently pelleted by centrifugation together with the beads and undissolved sample material. The purified lysate was mixed with the Binding Buffer and then applied to a new Microbial DNA column. The DNA that is bound to the column underwent a two-wash step. After a drying step, ready to use DNA could be eluted with Elution Buffer (5 mM Tris/HCl, pH 8.5).

· 16S rRNA and ITS Gene Sequencing

Libraries were prepared following the Illumina guidelines using Biome Makers custom primers for amplifying the 16S rRNA V4 region and the ITS1 region described previously (Becares & Fernandez, 2017). Sequencing was conducted in an Illumina MiSeq instrument using pair-end sequencing (2×300 bp).

· BeCrop® indexes

BeCrop® indexes are patented indicators to assess the health status of soils based on metagenomics data as described by (Acedo et al., 2022). Briefly, these indicators assess relevant traits related to soil health ranging from metabolic potential to biocontrol and hormones estimations. BeCrop indexes have been included in previous soil microbiome scientific studies (Milke et al., 2024). Becrop® indexes take scores among 1 and 5.

· Limits of acceptance

To measure the differences and similarities of the different DNA isolation kits we use:

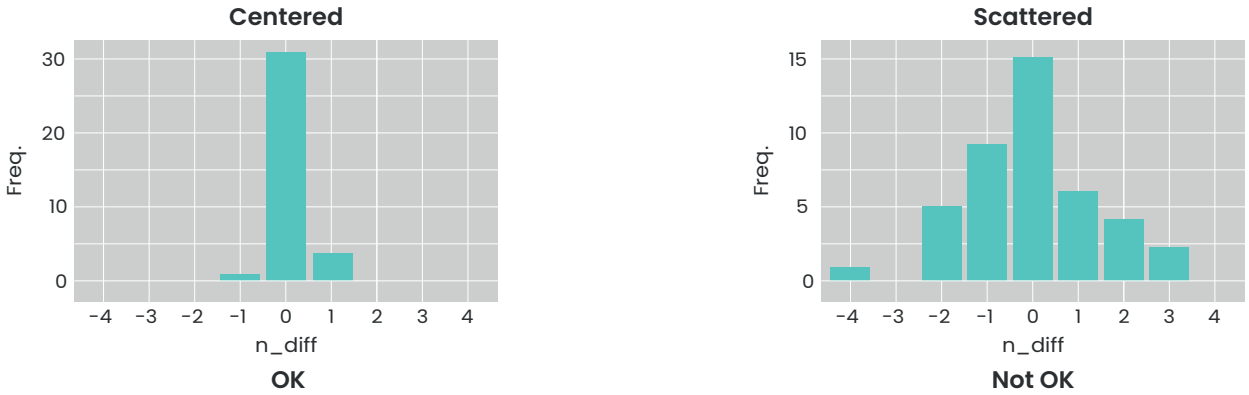
1. Taxonomy correlation Indicated by **Pearson Correlation**

Size of Correlation	Interpretation
.90 to 1.00 (-.90 to -.100)	Very high positive (negative) correlation
.70 to .90 (-.70 to -.90)	High positive (negative) correlation
.50 to .70 (-.50 to -.70)	Moderate positive (negative) correlation
.30 to .50 (-.30 to -.50)	Low positive (negative) correlation
.00 to -.0 (-.00 to -.30)	Negligible correlation

Limits of acceptance:

- Bacteria Marker (16S) ≥ 0.75
- Fungi Marker (ITS) ≥ 0.68

2. Ranks or Reports concordance (expressed in %)



Limits of acceptance:

- Difference 0 AND +1/-1 ≥ 80%

Results and discussion

· Taxonomy correlation

To measure the differences and similarities between both isolation kits, taxonomy correlation indicated by Pearson correlation was calculated. Figure 1 shows a high correlation of taxonomic abundances for 16S (0,98) and ITS (0,87) was observed between samples isolated with DanaGene kit and DNeasy kit, far above the established limits of acceptability (0,75 for 16S and 0,68 for ITS) established internally in BMK.

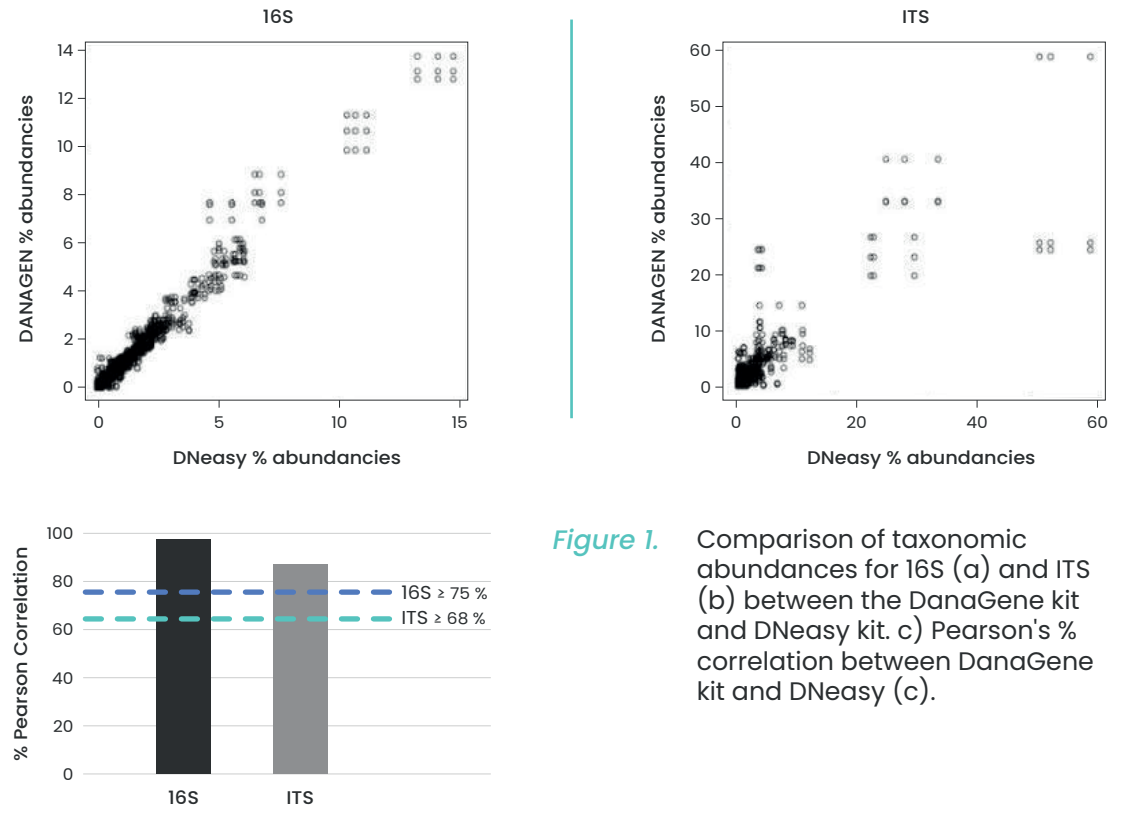


Figure 1. Comparison of taxonomic abundances for 16S (a) and ITS (b) between the DanaGene kit and DNeasy kit. c) Pearson's % correlation between DanaGene kit and DNeasy (c).

The analyses were extended to check similarity among replicates from DanaGene kit, where a high correlation of taxonomic abundances was also observed (0,99 for 16S and 0,81 for ITS) (Figure 2).

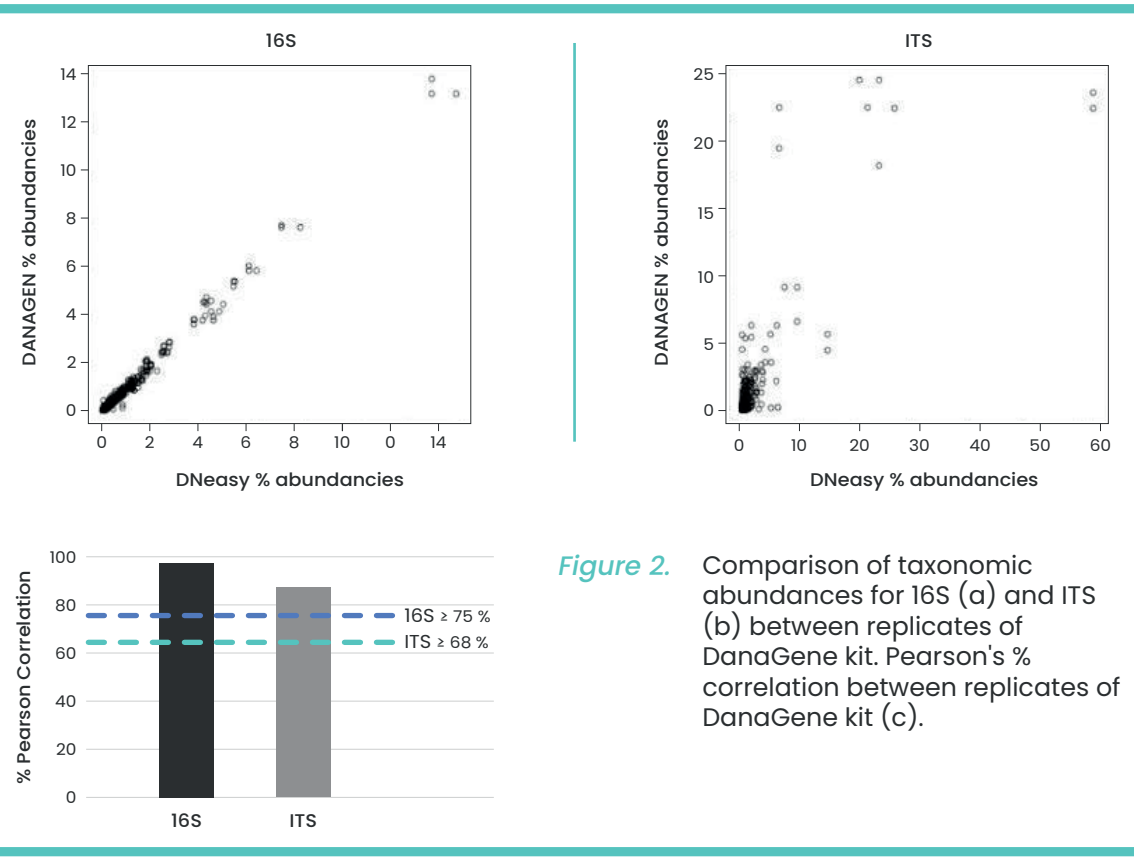


Figure 2. Comparison of taxonomic abundances for 16S (a) and ITS (b) between replicates of DanaGene kit. Pearson's % correlation between replicates of DanaGene kit (c).

· Concordance in BeCrop® indexes

After the taxonomic level comparison, this section presents the results at a higher abstract level, in terms of Becrop indices®. Figure 3 shows the difference between Becrop® score of the two isolation kits. In 86%, 95% and 99% of the cases, there were no differences or slightly low differences (+1 or -1) between the two isolation kits for the results obtained for the BeCrop® indices, classified among impacts, diseases and nutrients (panels a, b and c), respectively. A concordance greater than 80% reflects very high similarity (Figure 3.d).

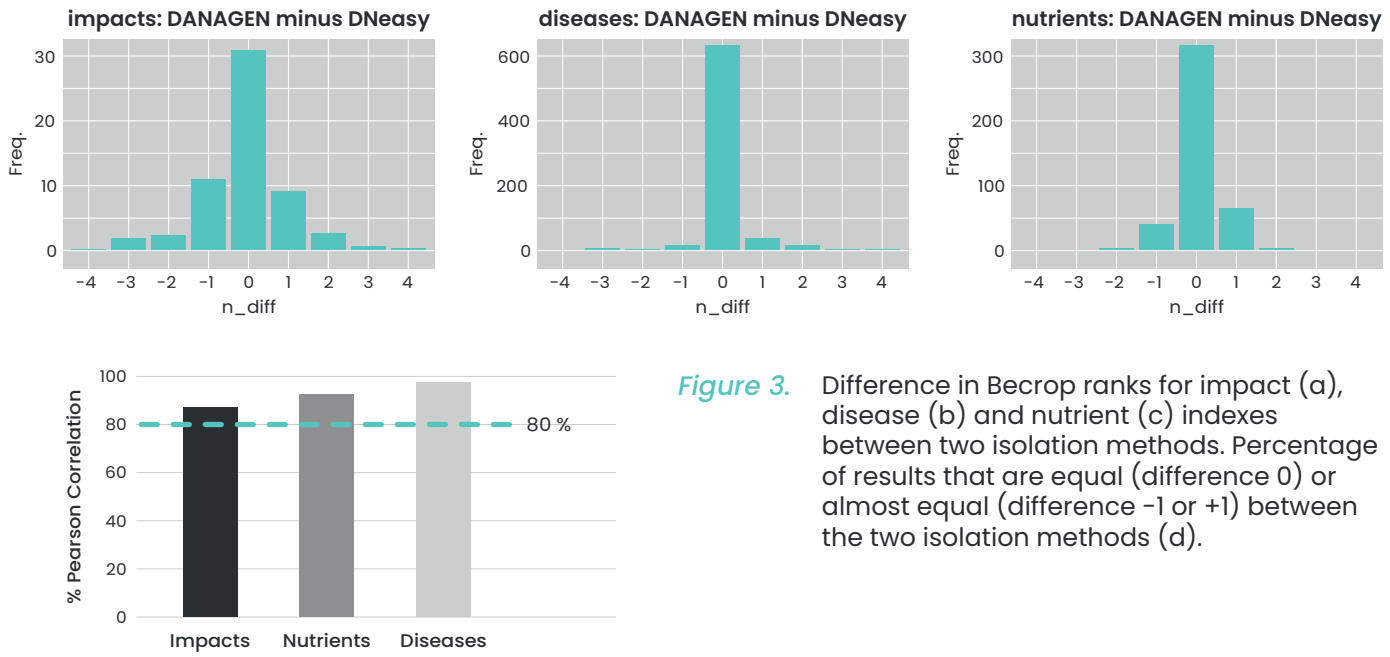


Figure 3. Difference in Becrop ranks for impact (a), disease (b) and nutrient (c) indexes between two isolation methods. Percentage of results that are equal (difference 0) or almost equal (difference -1 or +1) between the two isolation methods (d).

Conclusion

The new DANAGENE Microbiome Soil DNA kit method was compared to the gold standard in microbial soil sample prep, the DNeasy PowerSoil Pro Kit (QIAGEN).
The results of this study demonstrate that the new proposed DANAGENE Microbiome Soil DNA kit provides similar results in comparison with the gold standard in microbiome soil sample prep, so it can be used for an efficient and fast DNA isolation for microbiome and metagenomic soil analysis.

References

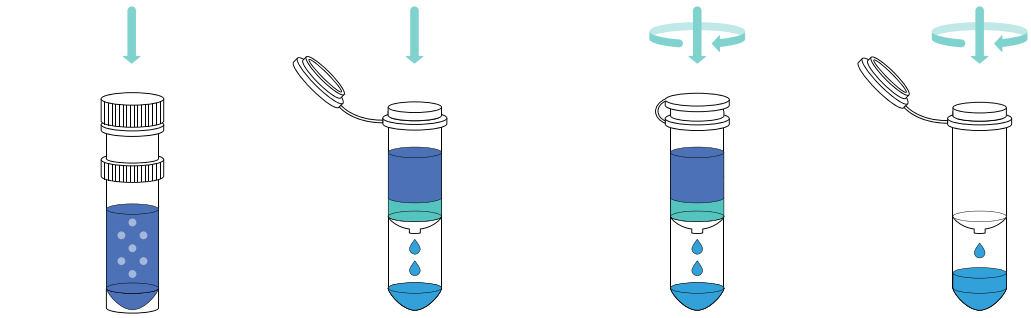
Becares A.A. and Fernández A.F. (2017) Microbiome Based Identification, Monitoring and Enhancement of fermentation. Processes and Products.W02017096385A1
Acedo A., Ortega-Arranz H., Almonacid D., Ferrero A. Methods and systems for generating and applying agronomic indices from microbiome-derived parameters. US202/0268756A1, 2022. p. 9.
Milke F., Rodas-Gaitan H., Meissner G., Masson V., Oltmanns M., Möller M., Wohlfahrt Y., Kulig B., Acedo A., Athmann M. and Fritz J. (2024) Enrichment of putative plant growth promoting microorganisms in biodynamic compared to organic agriculture soils. ISME Communications, ycae021. https://doi.org/10.1093/ismeco/ycae021.

DANAGENE MICROBIAL DNA KIT

DANAGENE Microbial DNA is designed for rapid purification of **highly pure genomic DNA from microorganisms (gram-negative and gram-positive bacteria, yeast, and fungi)**.

Microbial samples such as gram-positive bacteria, yeast, and spores can be difficult to lyse due to their strong complex cell wall structures. The DANAGENE Microbial DNA kit replaces enzymatic lysis by utilizing mechanical disruption of cell wall structures with the Bead Microtubes. The Bead Microtubes can be used in combination with many compatible disruptive devices.

Product workflow overview:



Specifications:

- Designed for rapid purification of highly pure genomic DNA from microorganisms (gram-negative and gram-positive bacteria, yeast and fungi).
- Silica-membrane technology with Mini Spin columns.
- Bead Microtubes for efficient lysis included in combination liquid Proteinase K.
- Suitable for a large variety of starting materials: Microbial cultures and agar plates.
- Sample material: 1.5 ml culture up to 50 mg wet weight cell pellet.
- Typical yield: Approx. 5-25 µg depends on sample type.
- Preparation Time: 35 min.
- Elution volume: 100 µl.

Ordering information:

Reference	Product Description	Preps
0619	DANAGENE MICROBIAL DNA KIT	50

DANASTOOL Sample Collection MICROBIOME Kit

DANAGEN-BIOTED has developed a complete system for processing samples of human or animal feces.

DANASTOOL Sample Collection MICROBIOME Kit enables collection, storage and stabilization of stool samples. It comes in a tube with spoon and liquid stabilization solution that preserves the MICROBIOME profiling.

DANASTOOL Sample Collection
MICROBIOME RUO Kit

REAL STOOL Sample Collection
MICROBIOME CE-IVD Kit

CE-IVD Marked version available:

- CE-IVDR marked in accordance with the European Commission Regulation (EU) No. 2017/746.
- Ideal for use in *in vitro* diagnostic workflows.

Specifications:

- Easy to use, designated for collection and safe transportation because the samples become Not infectious.
- It is not necessary to process the samples immediately.
- It stabilizes the DNA for several months at room temperature and at -20 or -80 indefinitely.
- Eliminate odour during processing.
- Compatible with a variety of purification systems. **The use of our DANAGENE MICROBIOME DANASTOOL DNA Kit is highly recommended**.
- The DANASTOOL preservative solution preserves microbiota profiles for unbiased and reproducible results and providing sample homogeneity eliminating sample variability.
- The DANASTOOL preservative solution and our DNA isolation method are capable of maintaining DNA integrity.

DANAGENE MICROBIOME DANASTOOL KIT

The **DANAGENE MICROBIOME DANASTOOL kit** has been designed for a fast and efficient purification of **microbial DNA** from:

A. Stool homogenate from 0.50-1.0 gr stool and stabilized in 8 ml DANASTOOL.

DANAGENE MICROBIOME FECAL DNA KIT

The **DANAGENE MICROBIOMEFecal DNA kit** is designed for the efficient isolation of **microbial DNA** from:

A. up to 250 µl fresh and frozen human or animal stool samples.

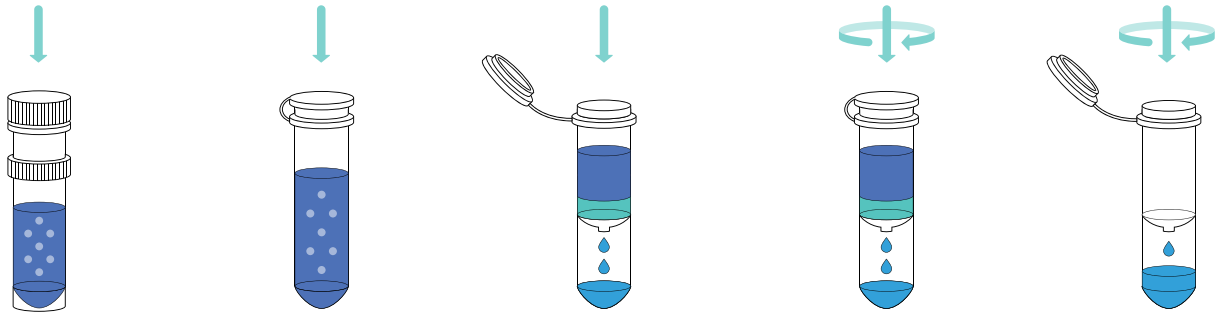
B. 250 µl preserved stool sample stabilized in DANASTOOL Sample Collection MICROBIOME Kit.

C. 400 µl preserved stool sample stabilized in DANASWAB Sample Collection MICROBIOME Kit.

Our **New DANAGENE Microbiome Fecal DNA kit** is even more effective than our original Microbiome Fecal technology and is designed to isolate high yields of pure **microbial DNA from stool samples for microbiome and metagenomic analysis**.

The kit features a novel **Microbial DNA Column** and optimized chemistry for a more efficient removal of PCR inhibitors (such as polysaccharides, heme compounds and bile salts) using a novel **Microbiome Lysis Buffer and Microbiome Precipitation Buffer**.

Product workflow overview:



Add sample and Lysis Buffer to Bead Tube and vortex to homogenize

Precipitate contaminants

Load supernatant for Binding DNA

Washing

Elution

Specifications:

- Designed for a fast and easy purification microbial DNA from different types of stool samples.
- Silica-membrane technology with news microbial DNA columns.
- Optimized lysis method-Combination of heat, chemical and mechanical lysis via bead-based homogenization enables isolation of DNA from yeast, fungi, Gram-negative and Gram-positive bacteria.
- Eliminates inhibitory substances, including lipids, polysaccharides and heme.
- Typical yield: Approx. 5-60 µg depends on sample type.
- Elution volume: 100-200 µl.
- No phenol/cloroform extraction or ethanol precipitation is necessary.

Application data:

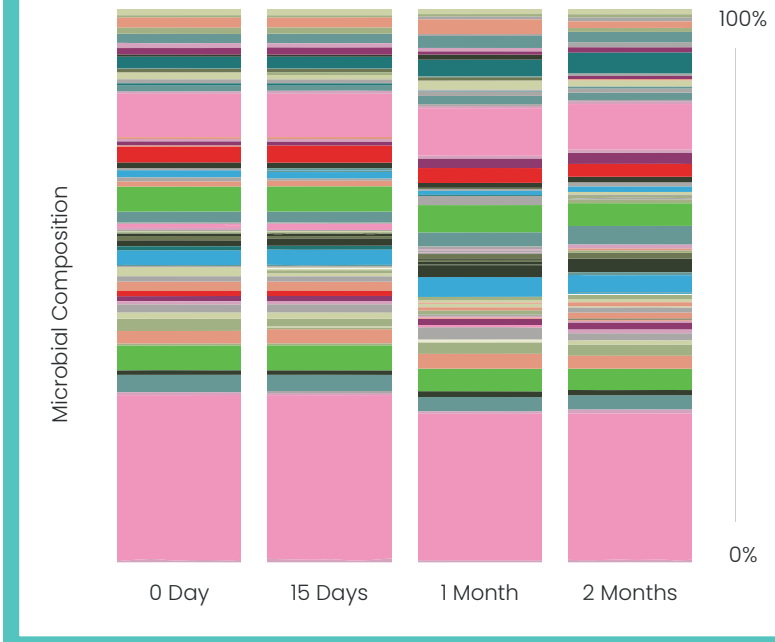


Figure 1.

Microbial composition of stool samples preserved at room temperature is unchanged after two months with DANASTOOL Sample Collection MICROBIOME Kit.

Stool samples were taken using our system and stored at room temperature. They were sampled at the indicated time points and processed with the DANAGENE MICROBIOME FECAL DNA Kit. The isolated DNA was the subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Samples had a constant microbial composition.

Ordering information:

Reference	Product Description	Preps
0620	DANAGENE Microbiome FECAL DNA Kit	50
0620.50 DANASTOOL	DANAGENE Microbiome DANASTOOL Kit	50
0620.250 DANASTOOL	DANAGENE Microbiome DANASTOOL Kit	250
0617	DANASTOOL Sample Collection MICROBIOME Kit	50
0618	DANASTOOL Sample Collection MICROBIOME Kit	250
0626.100	DANASWAB Sample Collection MICROBIOME Kit	100
RBMSC50DANCE	REAL STOOL Sample Collection MICROBIOME Kit CE-IVD	50

DANASALIVA Sample Collection MICROBIOME Kit

The microorganisms found in the human oral cavity have been referred to as the oral microflora, oral microbiota, or more recently as the **oral microbiome**.

The **oral microbiome** is one of the most diverse of any human-associated microbial community. The oral microbiome is a causative factor in conditions such as dental caries, periodontal disease, and halitosis, and has also been implicated as a reservoir for infection at other body sites and in the pathogenesis of non-oral diseases, such as inflammatory bowel disease.

DANAGEN-BIOTED has developed a complete system for the study of **ORAL MICROBIOME**:

1. DANASALIVA Sample Collection MICROBIOME Kit is An all-in-one collection kit for the collection and stabilization of microbial DNA from saliva.

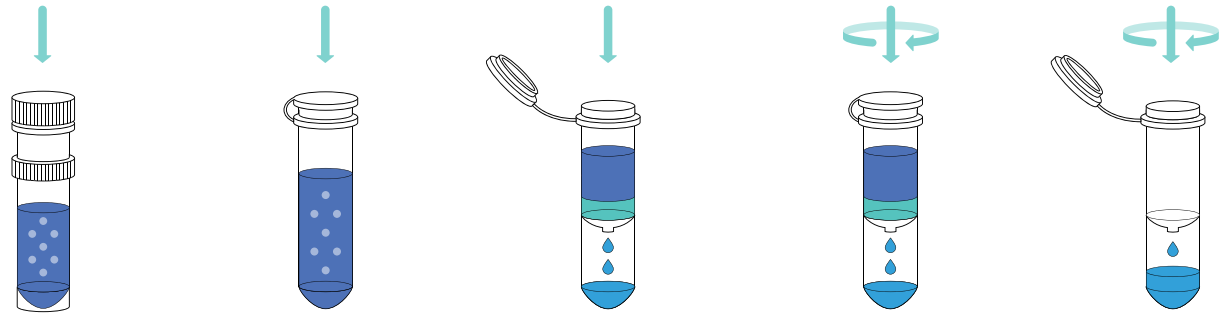
Specifications:

- All-in-one devices for optimal self-collection.
- Standardize sample collection.
- Stabilize microbial DNA at ambient temperature at least 1 year.
- Provide a snapshot of the saliva microbiome.
- Suitable for NGS downstream applications.

DANAGENE MICROBIOME SALIVA DNA Kit

2. DANAGENE MICROBIOME SALIVA DNA Kit has been designed for a fast and efficient purification of microbial DNA from saliva samples and preserved samples with our DANASALIVA Sample Collection MICROBIOME Kit.

Product workflow overview:



Add sample and Lysis Buffer to Bead Tube and vortex to homogenize

Precipitate contaminants

Load supernatant for Binding DNA

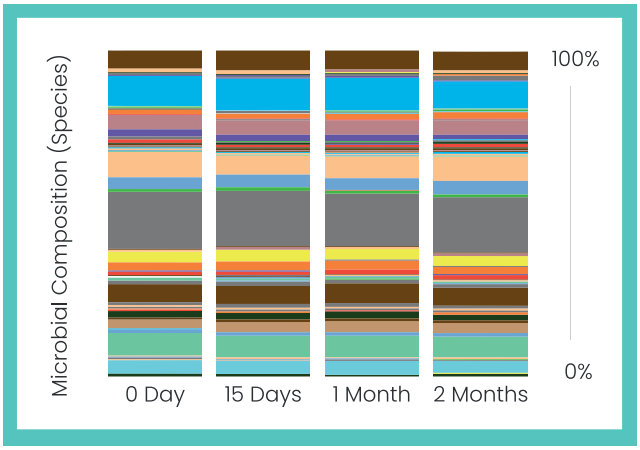
Washing

Elution

Specifications:

- Designed for rapid purification of highly pure microbial DNA for microbiome analysis.
- Silica-membrane technology with Mini Spin columns.
- Bead Microtubes for efficient lysis included in combination liquid Proteinase K.
- Sample material: saliva / preserved saliva samples.
- Typical yield: Approx. 2-20 µg depends on patient.
- Preparation Time: 35 min.
- Elution volume: 100 µl.

Application data:



Microbial composition of saliva sample preserved at room temperature is unchanged after two months with DANASALIVA Sample Collection MICROBIOME Kit.

Saliva samples were taken using our system and stored at room temperature. They were sampled at the indicated time points and processed with the DANAGENE MICROBIOME SALIVA DNA Kit. The isolated DNA was subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Samples had a constant microbial composition.

Ordering information:

Reference	Product Description	Preps
0603.45100	DANASALIVA Sample Collection MICROBIOME Kit	100
0603.45500	DANASALIVA Sample Collection MICROBIOME Kit	500
0603.451000	DANASALIVA Sample Collection MICROBIOME Kit	1000
0623	DANAGENE MICROBIOME saliva DNA Kit	50

VAGINAL SELF-COLLECTION SWAB

The **vaginal microbiome** is a specific compartment of the human microbiome. Unique conditions of the vagina are characterized by a few microbial species, usually lactobacilli.

The vaginal microbiome harbours diverse communities of microorganisms, known as vaginal flora which has an important impact on women's health as well as that of their new-borns.

The composition of the vaginal microbiota depends on age, menstruations, hormonal fluctuations, sexual behaviours, and also the use of drugs such as probiotics and antibiotics causing its imbalance.

As part of the human microbiome project, the study of the **vaginal microbiome** has shown a relationship between bacteria present in the vagina and diseases.

DANAGEN-BIOTED has developed a complete system for the study of **VAGINAL MICROBIOME**:

1. VAGINAL SELF-COLLECTION SWAB for the collection and stabilization of microbial DNA from vagina for microbiome analysis.

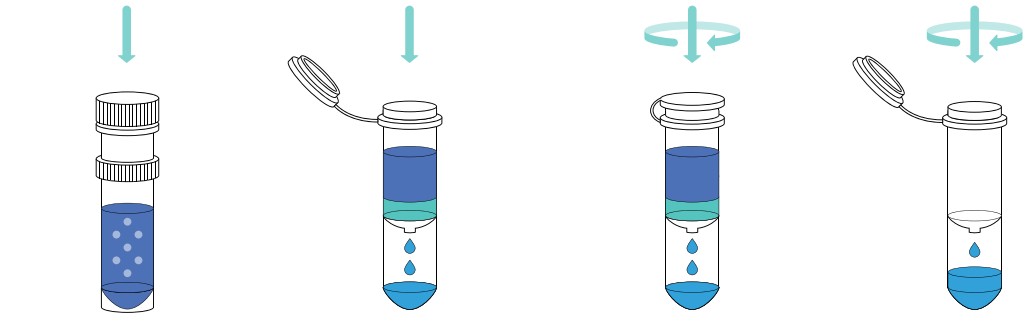
Specifications:

- Easy to use.
- Made of nylon FLOQSwabs® for soft collection.
- Round shape design to reduce discomfort during the collection.
- Designed to standardize the collection (one time right, no repeated sampling).
- Ergonomic shaft to facilitate the rotatory movement.
- Red mark to show fingers position.
- Ability to preserve **nucleic acids stability up to 4 weeks at room temperature**.
- The new solution for home self-sampling, collection, shipping and easy processing in the laboratory.
- The peel able barcode on tube label ensures efficient and **straightforward sample tracking**.

DANAGENE MICROBIOME VAGINAL DNA KIT

2. DANAGENE MICROBIOME VAGINAL DNA Kit has been designed for a fast and efficient purification of microbial DNA from vaginal swab samples.

Product workflow overview:



Add sample and Lysis Buffer to Bead Tube and vortex to homogenize

Load supernatant for Binding DNA

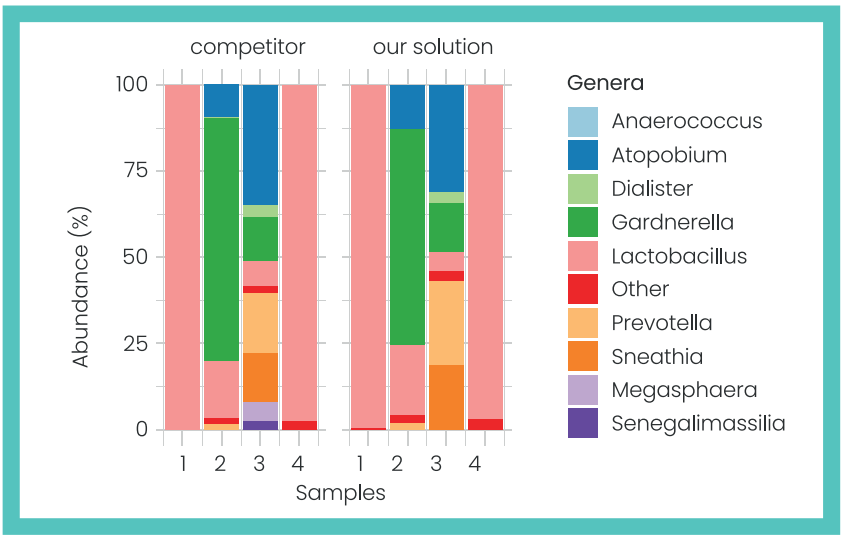
Washing

Elution

Specifications:

- Designed for rapid purification of highly pure microbial DNA for microbiome analysis.
- Silica-membrane technology with Mini Spin columns.
- Bead Microtubes for efficient lysis included in combination liquid Proteinase K.
- Sample material: vaginal swabs samples.
- Typical yield: it depends on patient.
- Preparation Time: 35 min.
- Elution volume: 100 µl.

Application data:



Bar plot showing the taxonomic composition, excluding unclassified reads, of our analysis and the analysis performed on the same samples by a competitor.

Ordering information:

Reference	Product Description	Preps
0624	DANAGENE MICROBIOME VAGINAL Kit *	50

*Kit contains the Vaginal self-collection swabs and the reagents for the DNA isolation

DANASWAB Sample Collection MICROBIOME KIT

DANASWAB Sample Collection MICROBIOME Kit is designed **for the collection, ambient storage and transport from samples collected using a swab** wherever a swab may be deemed appropriate per application.

MICROBIOME Stabilization Solution take a microbial snapshot of a sample while inactivating pathogens making samples safe and ready for transport. Samples stored in the these microtubes are stable at ambient temperature, and can be frozen for longer-term storage.

Each collection microtube is pre-filled with 2 ml and the nucleic acid content of **samples is preserved at ambient temperature (DNA up to 1 year; RNA up to 15 days)**. For longer periods of time the samples must be frozen (-20 / -80 ° C).

To use these swab collection tubes, just swab any sample and break the tip into the collection device prefilled with the **MICROBIOME Stabilization Solution**.

Specifications:

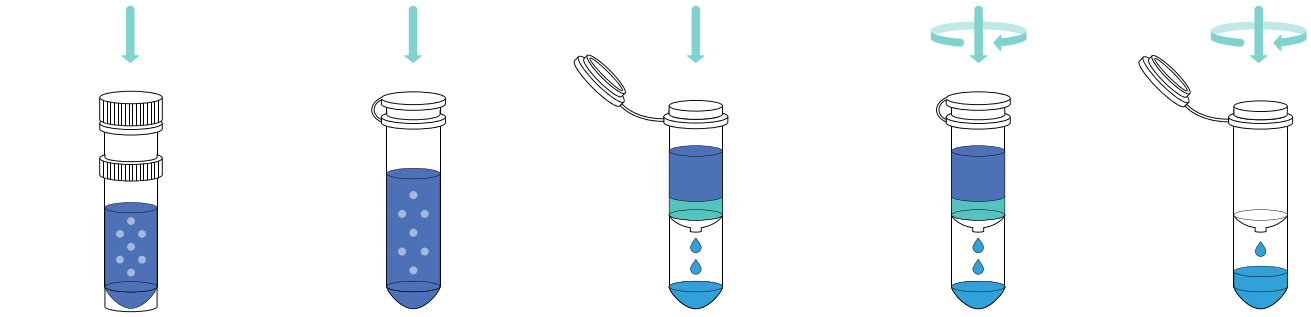
- Used for collection, storage and transportation of swab samples for MICROBIOME analysis.
- Inactivate microorganisms and viruses for safe and easy transport and handling.
- Compatible with many sample types, including nose, mouth, stool, vaginal, biological fluids, environmental samples.
- Room temperature transport.
- Preserve total DNA, including viral DNA, at room temperature (4°-25°C) 1 year.
- Preserve total RNA, including viral RNA, at room temperature (4°-25°C) 15 days.
- Compatible with most DNA and RNA isolation methods.

We recommend to use our DANAGENE SWAB MICROBIOME KIT for microbiome analysis and the DANAGENE MICROBIOME RNA Kit for RNA isolation

DANAGENE MICROBIOME SWAB DNA KIT

The DANAGENE MICROBIOME Swab DNA kit has been designed for a fast and efficient purification of **microbial DNA from preserved samples using our DANASWAB Sample Collection MICROBIOME Kit for microbiome analysis**.

Product workflow overview:



Add sample and Lysis Buffer to Bead Tube and vortex to homogenize

Precipitate contaminants

Load supernatant for Binding DNA

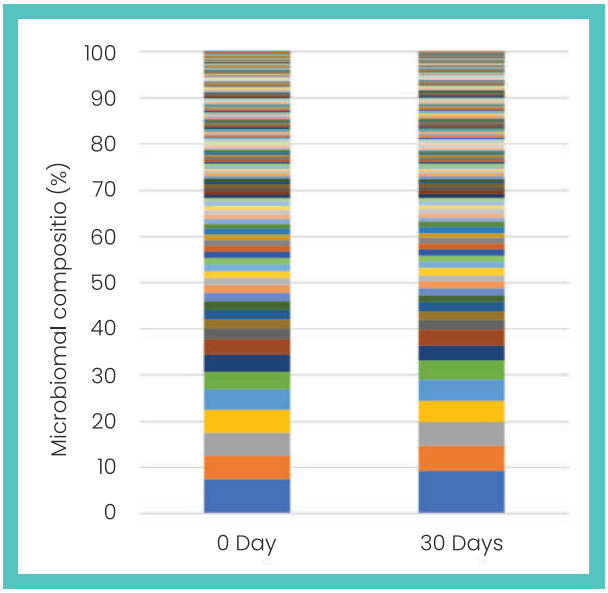
Washing

Elution

Specifications:

- Designed for a fast and easy purification microbial DNA from preserved samples using our DANASWAB Sample Collection MICROBIOME Kit
- Optimized lysis method-combination of heat, chemical and mechanical lysis via bead-based homogenization enables isolation of microbial DNA for microbiome analysis.
- Eliminates inhibitory substances.
- No phenol/chloroform extraction or ethanol precipitation is necessary.

Application data:



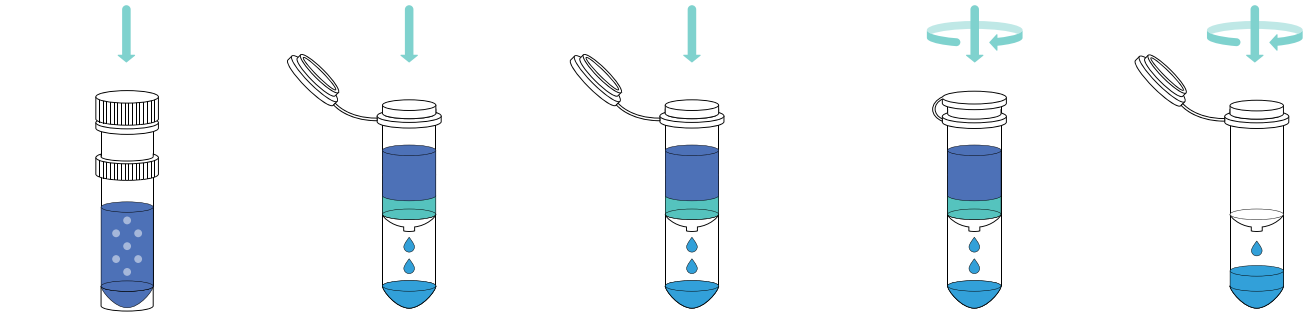
The microbiome composition and abundance of stool samples conserved 0 and 30 days in DANASWAB Sample Collection MICROBIOME Kit designed to preserve DNA and RNA do not present significant differences. The composition of both samples is constant

DANAGENE MICROBIOME RNA KIT

The DANAGENE MICROBIOME RNA kit has been designed for an efficient purification of **Microbiome RNA (bacterial, fungal, protozoan, algae, viral and host RNA) from preserved samples using our DANASWAB Sample Collection MICROBIOME Kit for microbiome analysis**.

Our DANASWAB Sample Collection Microbiome Kit allows to collect & preserves from a wide array of samples inputs (feces, soil, biological fluids and others swab samples).

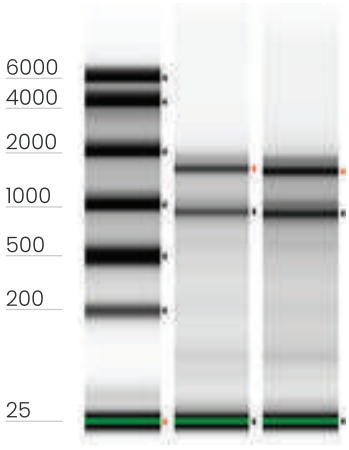
Product workflow overview:



Specifications:

- Designed for an easy purification of Microbiome RNA from preserved samples using our DANASWAB Sample Collection MICROBIOME Kit.
- Efficient lysis method ensures complete lysis of the microbial cell walls and accurate microbial analysis, free of bias.
- Total RNA (including small/micro RNAs) is inhibitor-free.

Application data:



Microbial RNA isolated with the DANAGENE MICROBIOME RNA kit. RNA was isolated from aliquots of 200µl of preserved feces samples with our DANAGENE MICROBIOME RNA kit. These samples were preserved at room temperature for 7 days. Quality was assessed using the Agilent 4150 TapeStation.

Ordering information:

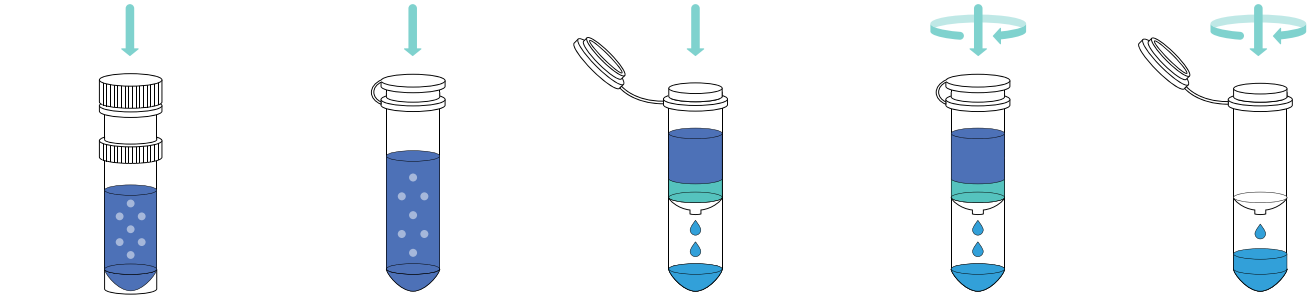
Reference	Product Description	Preps
0626.100	DANASWAB Sample Collection MICROBIOME Kit	100
0625	DANAGENE MICROBIOME SWAB DNA KIT	50
0622	DANAGENE MICROBIOME RNA KIT	50

DANAGENE MICROBIOME SOIL DNA KIT

The new **DANAGENE MICROBIOME Soil DNA kit** has been designed for the isolation of high molecular weight genomic DNA from microorganisms like Gram positive and Gram negative bacteria, archaea and fungi **from environmental samples of all soil types**. The kit uses a new **Microbial DNA Columns, Microbiome Lysis Buffer** and Microbiome Precipitation Buffer specifically designed for use with environmental samples containing high humic substances content, including difficult soil types.

In this procedure, the microorganisms are efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads. Proteins and Inhibitors are eliminated by precipitation using a new proprietary inhibitor removal buffer and subsequently pelleted by centrifugation together with the beads and undissolved sample material. The purified lysate is mixed with the Binding Buffer and then applied to a Microbial DNA column. The DNA that is bound to the column undergoes a two wash step. After a drying step, ready to use DNA can be eluted with Elution Buffer (5 mM Tris/HCl, pH 8.5).

Product workflow overview:



Add sample and Lysis Buffer to Bead Tube and vortex to homogenize

Precipitate contaminants

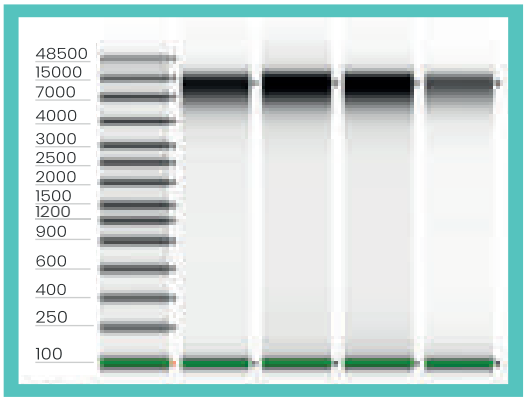
Load supernatant for Binding DNA

Washing

Elution

Specifications:

- Designed for a fast and easy purification microbial DNA from different types of soil samples.
- Optimized lysis method-combination of heat, chemical and mechanical lysis via bead-based homogenization enables isolation of DNA from archaea, fungi, Gram-negative and Gram-positive bacteria.
- Eliminates inhibitory substances as humic substances and others inhibitors.
- No phenol/chloroform extraction or ethanol precipitation is necessary.
- Typical yield: Approx. 1-20 µg depends on sample type.
- Preparation Time: 35 min.
- Elution volume: 50-100 µl.
- The eluted DNA is ready to use for all standard downstream applications. In most cases the concentrated DNA can be used as PCR template without further dilution for highest sensitivity.



Application data:

Efficient isolation of DNA from soil samples. DNA was isolated from 4 different soil samples uisng our DANAGENE MICROBIOME SOIL DNA Kit. Quality was assessed using the Agilent 4150 TapeStation.

Ordering information:

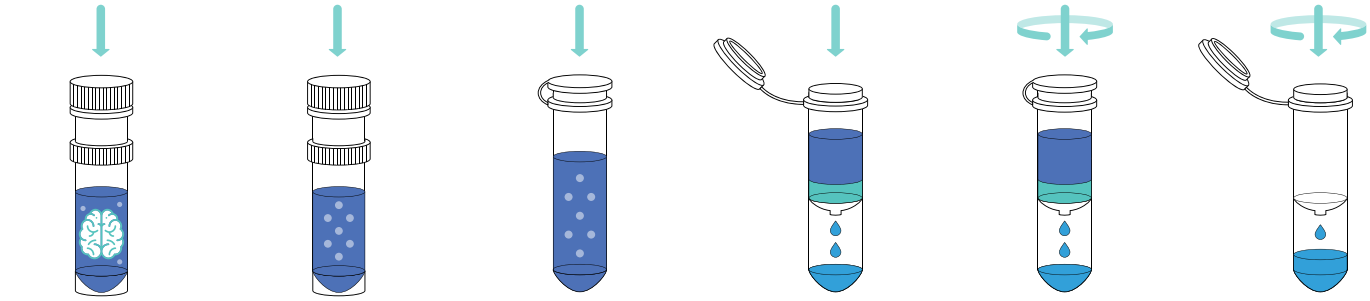
Reference	Product Description	Preps
0621	DANAGENE MICROBIOME SOIL DNA Kit	50

DANAGENE MICROBIOME TISSUE DNA KIT

The DANAGENE Microbiome Tissue DNA kit is designed for the efficient isolation of **microbial DNA from tissues samples or biopsies**.

In this procedure, first the tissue samples are lysed by a special Tissue Lysis Buffer, after microorganisms are efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads. Proteins and Inhibitors are eliminated by precipitation using a new proprietary inhibitor removal buffer and subsequently pelleted by centrifugation together with the beads and undissolved sample material. The purified lysate is mixed with the Binding Buffer and then applied to a Microbial DNA column. The DNA that is bound to the column undergoes a two wash step. After a drying step, ready to use DNA can be eluted with Elution Buffer (5 mM Tris/HCl, pH 8.5).

Product workflow overview:



Add tissue sample and Tissue Lysis Buffer. Incubate with proteinase K

Add lysate and Microbiome Lysis Buffer to Bead Tube and vortex to homogenize

Precipitate contaminants

Load supernatant for Binding DNA

Washing

Elution

Specifications:

- Designed for a fast and easy purification microbial DNA from different types of stool samples.
- Silica-membrane technology with news microbial DNA columns.
- Optimized lysis method-Combination of heat, chemical and mechanical lysis via bead-based homogenization enables isolation of DNA from yeast, fungi, Gram-negative and Gram-positive bacteria.
- Eliminates inhibitory substances, including lipids, polysaccharides and heme.
- Typical yield: Approx. 5-60 µg depends on sample type.
- Preparation Time: 35 min.
- Elution volume: 100-200 µl.
- No phenol/cloroform extraction or ethanol precipitation is necessary.

Ordering information:

Reference	Product Description	Preps
0627	DANAGENE MICROBIOME TISSUE DNA Kit	50

cancers

MDPI

Article

New Insights into Mucosa-Associated Microbiota in Paired Tumor and Non-Tumor Adjacent Mucosal Tissues in Colorectal Cancer Patients

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Simple Summary: Colorectal cancer (CRC) is a major burden of disease worldwide. Increasing scientific evidence highlights the role of the gut microbiota in the initiation, development and treatment of CRC. Currently, the analysis of CRC-associated gut microbiota has several limitations that hinder its implementation in precision medicine, including selection of sample type, sequencing platform and taxonomic classification. This article aims to address these constraints to provide data on CRC-associated microbiota and facilitate the implementation of its analysis in personalized medicine. To this end, mucosa-associated microbiota from paired tumor and non-tumor adjacent tissue samples from 65 CRC patients was analyzed through V3–V4 region of 16S rRNA gene amplification, MinION sequencing and NCBI taxonomic classification. Results consistent with available evidence have been obtained. Moreover, to our knowledge, this is the first study that identifies the possible association between a higher relative abundance of *Streptococcus periodonticum* and a lower relative abundance of *Corynebacterium* with CRC.

Citation: González, A.; Fullaondo, A.; Navarro, D.; Rodriguez, J.; Tirnauca, C.; Odriozola, A. New Insights into

Danagen-Bioted is an expert in the development of molecular biology and biotechnology kits both for use in research and for the teaching of life sciences.


DANAGEN
Life Science Research
INNOVATIVE SME
GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD

DNA and RNA preservation systems for biological sample collection, storage, transportation without the requirement of refrigeration and downstream purification.

Why is it necessary to stabilize samples between collection and analysis?

To obtain a truly accurate outcome, a sample must be stabilized and protected from the point of collection until it is analysed downstream.

Sample stabilization is essential because if a biospecimen experiences any changes between collection and analysis, the sample may not yield enough quality DNA to conduct analysis. Data that is generated and analysed from a changed biospecimen will not be a true representation of the in vivo state of the donor, leading to flawed and irreproducible results.

The longer a sample remains unstabilized, the greater the likelihood that it will change as a result of environmental impacts and natural biological degradation.

Other factors that can cause post-collection changes in an unprotected sample include the rate of freezing, the size of aliquots, storage duration, the type of and time in stabilizing reagent and user/handler error.

Freezing your samples can potentially protect and stabilize them, but not without accompanying costs, complexity and risks.

What does it mean for a sample to be stabilized?

Sample stabilization provides:

- **Reproducibility and Sample Homogeneity** – DNA yield, quality and performance are the same across multiple aliquots taken from the sample at different time points.
- **DNA Integrity** - prevents DNA degradation ensuring that the DNA remains of a molecular weight suitable for all downstream applications.
- **Prevention of microbial growth**.

Essentially, stabilization ensures that your DNA samples does not change over time. Without stabilization, the sample may not yield enough quality DNA to perform downstream assays.

Just what is meant by “immediate” stabilization?

When we say that a sample should be stabilized immediately, we mean within seconds of collection. Collection kits and stabilizing reagents should enable you to stabilize your samples as quickly as possible before any contamination or putrefaction can occur.

In the field of human genetics, not immediately stabilizing your samples can lead to wasted or lost samples, DNA degradation, and flawed or irreproducible results—an assay’s performance can be significantly affected by the introduction of variables to a biospecimen.

A stabilized sample is truly representative of the in vivo state of the donor, allowing you to have complete confidence in the accuracy of your discoveries.

Using saliva as the ideal sample

Saliva is one of the most accessible of our body's bio-fluids making saliva sample collection easy and non-invasive. Saliva also harbours a wide spectrum of genetic data that can be used for genetic research and clinical diagnostic applications.

Over the past few years, saliva has become recognized as a very important and reliable alternative to blood samples for genetic research, clinical diagnostics, personalized medicine and more.

What exactly is it that makes saliva such a good alternative to blood for genetic applications?

- DNA in saliva is derived from both buccal epithelial cells and white blood cells.
- The vast majority of DNA from saliva is of human origin. (average only 11.8% bacteria).
- Saliva yields high quantity high-molecular weight DNA.
- Saliva can reliably replace blood for DNA analysis.

Blood collection for genomic DNA presents several disadvantages: it is invasive and inconvenient for the donor, requires a trained medical professional, must be refrigerated for transportation and storage, and is difficult to transport across borders. All these factors can add significant costs to any genetic study and impact compliance rates.

It's really very similar. The DNA that you get from saliva is the same DNA that you get from blood. You will see similar 260/280 ratios and similar molecular weight."

Our sample-stabilizing products have been designed to be as easy-to-use as possible to lessen the likelihood of user error.

Each of our collection kits and reagents contain a chemistry that allows you to immediately stabilize a biological sample so that it cannot be affected by external factors, and all desired biological material is protected over time and temperature.

Purification of High-quality DNA from Saliva Samples with DANAGENE Saliva System applied to TargetSeq-NGS protocols

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Introduction

DANAGEN-BIOTED S.L has developed a method for the collection, stabili- zation, transportation and purification of DNA from saliva samples using the DANASALIVA Sample Collection Kit, a cost-effective collection and trans- portation device that effectively stabilizes buccal cells and white blood cells found in saliva over 1 year at room temperature. Then, saliva DNA is isolated from the preserved saliva samples via DANAGENE Saliva Kit. Here, it is dem- onstrated the efficiency genomic DNA samples extracted with DANAGENE Saliva System to prepare a custom library (HC-Gen Test) designed by AC- Gen Reading Life Inc. for Hereditary Cancer Diagnostic (http://www.ac-gen. com/hereditary-cancer).

Material and Methods

· Genomic Isolation, DNA quality and quantification

2ml saliva sample was collected using the DANASALIVA Sample Collection Kit from a hereditary colon cancer patient and gDNA was isolated from 600ul of saliva sample following DANAGENE Saliva Kit protocol. A fluorometry-based DNA method was used to accurately quantify DNA starting material. Size distribution of each DNA preparation was verified by gel electrophoresis and DNA purity was measured with a UV-Vis Spectrophotometer.

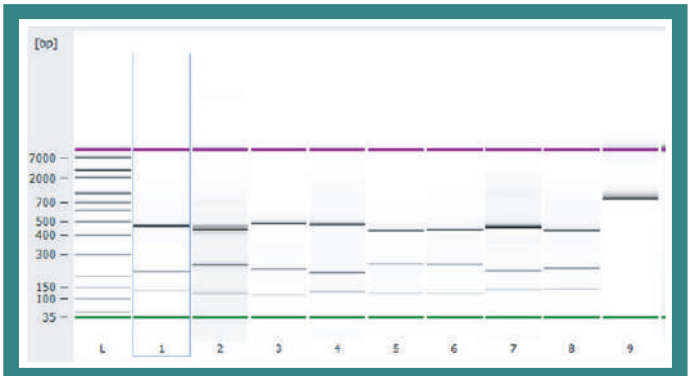

Figure 1.

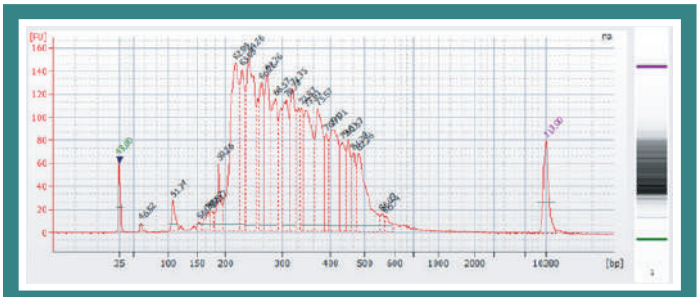

Figure 2.

High sensitivity electrophoretic analysis showed a perfect restriction reaction for the eight combinations of restriction enzymes (Figure 1) and a correct library profile (Figure 2).

· Library Target-Seq Preparation

gDNA sample was diluted to a final concentration of 5 ng/uL. 225ng of gDNA were digested in eight different restriction reactions, each containing two restriction enzymes. Restriction digestion reaction was validated by electrophoretic analysis of each sample. Then, all eight-digestion reactions corresponding to each DNA sample was transferred into appropriate tube. Next, biotinylated probes and barecode primers cassette were added and ligated. All interest target regions, corresponding with exonic regions of 37 genes related with Hereditary Cancer were captured using streptavidin-coated magnetic beads. Library was amplified and quantified for equimolar dilution. A pool of libraries were performed before proceeding to DNA sequencing with Ion PGM 200pb sequencing protocol using a 316V2 chip.

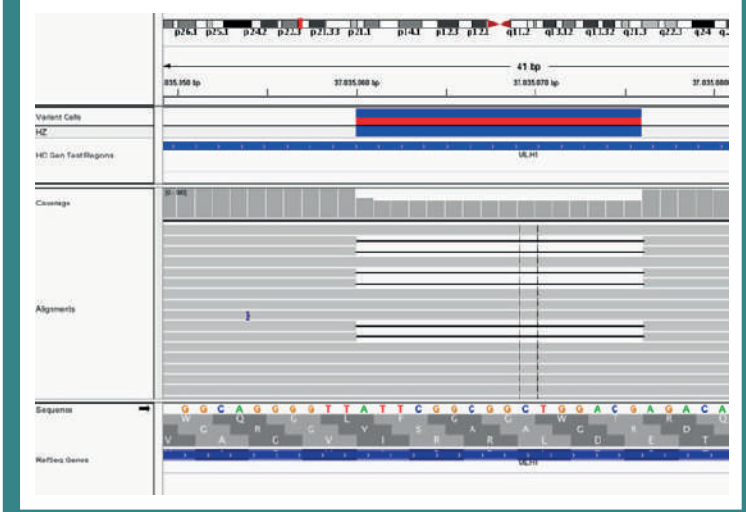

Figure 3.

Variant calling of DNA sequences obtained in PGM system per- fectly detected the frameshift mutation c.22_37del (p.I8Rdf*4) in MLH1 gene (Figure 3). This mutation had been previously analysed through Sanger sequencing for the same sample and it's related with Lynch syndrome.

Results

DNA yield and quality from saliva sample purified using DANAGENE Saliva kit are shown in Table 1.

Gel electrophoresis performed to check gDNA integrity, didn't show smearing below 2,5kb, indicating no sample degradation.

Conclusion

As when building a house, any good NGS experiment is founded in a proper starting material. In this paper it has been demonstrated the high quality of DANAGENE Saliva System kit for the isolation of gDNA applied to mutation screening of clinically important DNA variants with NGS technologies. DNA isolation from saliva samples it s a cost-effective method because samples can be collected directly for doctors without intervention of specially trained nurses and transported without special conservation requirements.

Day 0	Day 30	Day 30	Day 30
238	238	238	238
22978	26714	26714	26714

A method for preserving buccal swabs samples for gDNA integrity

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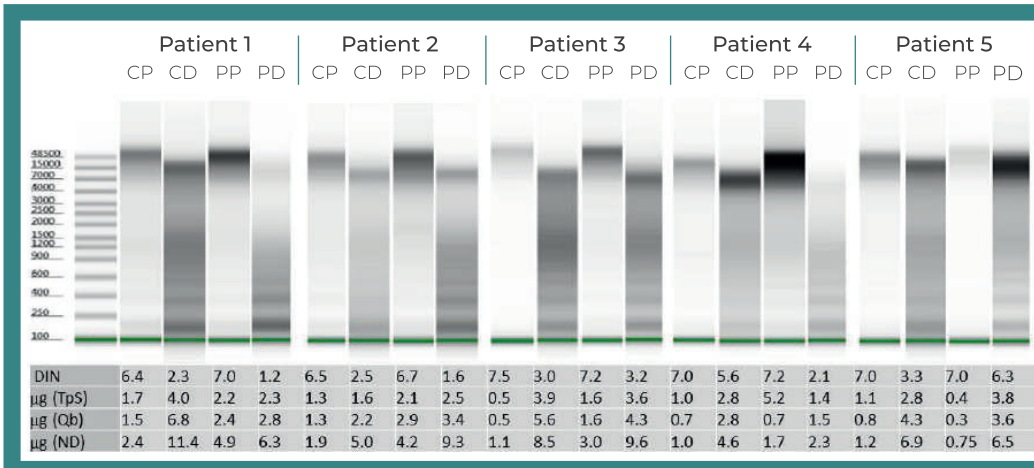
Introduction

The use of buccal swabs for non-invasive sample collection is well established. Samples can be stored for up to 2 weeks at 4°C before processing without a noticeable loss in DNA yield or quality. This storage condition is not often possible to apply immediately. If unprocessed samples are stored at room temperature, the bacteria and nucleases present in the buccal swabs will cause DNA degradation.

DANAGEN-BIOTED has developed a method using the DANASWABS Sample Collection Kit that contains a stabilizing buffer designed to completely stabilize the buccal cells from buccal swabs samples by inhibiting all enzymatic and microbial activity that occurs following any buccal sampling.

This system allows the release of the cells captured by the swab into a proprietary cell stabilizing buffer. Samples preserved with this system are stable for 1 year at room temperature so that they can be transported safely to the laboratory for processing.



DIN	6.4	2.3	7.0	1.2	6.5	2.5	6.7	1.6	7.5	3.0	7.2	3.2	7.0	5.6	7.2	2.1	7.0	3.3	7.0	6.3
µg (TpS)	1.7	4.0	2.2	2.3	1.3	1.6	2.1	2.5	0.5	3.9	1.6	3.6	1.0	2.8	5.2	1.4	1.1	2.8	0.4	3.8
µg (Qb)	1.5	6.8	2.4	2.8	1.3	2.2	2.9	3.4	0.5	5.6	1.6	4.3	0.7	2.8	0.7	1.5	0.8	4.3	0.3	3.6
µg (ND)	2.4	11.4	4.9	6.3	1.9	5.0	4.2	9.3	1.1	8.5	3.0	9.6	1.0	4.6	1.7	2.3	1.2	6.9	0.75	6.5

Figure 1. CP: COPAN Swab, preserved ; CD: COPAN Swab, direct ; PP: PURITAN Swab, preserved ; PD: PURITAN swab, direct.

Material and Methods

Buccal cells samples were collected from 5 patients using our DANASWABS Sample Collection Kit. Two swabs from different brands previ- ously evaluated as the best performers were used (data not shown).

4 samples were taken from each patient on different days and at the same time, 2 samples were preserved with our buffer and the other 2 unpreserved (one per swab type).

3 days after sampling, the samples were processed for DNA isolation following DANAGENE Swabs DNA Kit protocol and were analyzed using the Agilent 4200 TapeStation System.

Results

DNA yield, quality and integrity were tested using the Qubit (Qb), Nanodrop (ND) and TapeStation Instrument (TpS) respectively.

Conclusion

The gel image from TapeStation and quantifications with Qubit and Nanodrop show that the DNA yield of DNA isolations performed from unpreserved swabs is higher than from swabs preserved using our system, but while the DNA of unpreserved samples is extensively degraded, preserved samples exhibit DNA integrity. The TapeStation allows to calculate the DIN, a numerical assessment of gDNA integrity referred as the DNA integrity number (DIN).

Better results are also obtained with preserved samples in quantitive PCR assays (data not shown).

In this paper it is demonstrated that the buccal cells are stabilized using our DANASWABS Sample collection Kit, with the structure and integrity of the DNA being fully maintained for further downstream processing applications.

BioTechniques 61:153 (September 2016) doi 10.2144/000114455

Saliva as a sample type for genomic applications

DANASALIVA Sample Collection Kit
DANAGENE SALIVA DNA Kit
DANAGENE SPIN SALIVA DNA Kit

Introduction

Saliva collection provides a non-invasive alternative source of genomic DNA for use in genetic analysis.

Saliva offers several appealing features for both researchers and clinicians. It is a non-invasive and needle-free method, that still enables collection of a high quantity of white blood cells, source of high-quality DNA. The collection can be performed unsupervised, and the ability to store and transport the collected samples at room temperature makes it an attractive choice for wide range of genetic analysis projects.

A very similar workflow in the lab can be used to extract DNA from saliva as is used for blood, producing the same resulting genomic DNA, with similar 260/280 ratios and similar molecular weight.
In this application note we evaluate the different DANAGEN solutions for working with saliva samples:

- The **DANASALIVA Sample Collection Kit** is specifically designed for collecting and preserving saliva samples for long periods of time at room temperature.
- The **DANAGENE Saliva DNA kit** is designed to isolate genomic DNA from fresh or preserved saliva samples using a "salting-out" method.
- The **DANAGENE SPIN Saliva DNA kit** is designed to isolate genomic DNA from preserved saliva samples using a MicroSpin column method.

Figure 1. DANASALIVA Sample Collection Kit/DANAGENE Spin Saliva DNA Kit

Material and Methods

· Saliva sample collection

A saliva sample of 2 ml was collected from 2 healthy donors using our DANASALIVA Sample Collection Kit (Fig.1) and stored at room temperature for several days.

· Manual Human Genomic DNA extraction for PCR assays

Method 1.
Human genomic DNA was isolated from 400 µl of preserved saliva sample following the specific protocol of **DANAGENE Saliva DNA kit**.

Method 2.
Human genomic DNA was isolated from 400 µl of preserved saliva sample following the specific protocol of **DANAGENE SPIN Saliva DNA kit** (Fig.1).

For each donor, three samples were processed.

· Automated Human Genomic DNA extraction for PCR assays

Method 3.
Human genomic DNA was isolated from 200 µl of preserved saliva sample using the ZiXpress Whole Blood Genomic DNA Extraction Kit using the **ZiXpress32 Robot** (ZINEXTS,Taiwan) (Fig.2) with the following modifications:

A. For the reagent plate preparation, we used the Viral Lysis Buffer (DANAGEN-BIOTED,Spain) instead of the Lysis buffer A.

B. For the robot protocol extraction setting, we edited a new protocol called SALIVA. We only changed the lysis buffer incubation time (10 minutes instead of 20 minutes) from the existing default blood protocol on the robot.

For each donor, four samples were processed.

Figure 2. ZiXpress 32 Robot

· Quality and Quantification of extracted DNA

For DNA quantification, DNA concentration was determined fluorometrically on the Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) using the QUBIT dsDNA BR Assay Kit.

For DNA quality, DNA purity was determined via 260/280 and 260/230 ratios measured on the NanoDrop (Thermo Fisher Scientific, USA).

· Gel Electrophoresis

For visual analysis of Human DNA and PCR size and integrity, 20 µl of DNA from the final elution were loaded onto a 1% agarose TAE gel and run for 30 minutes at 125 V.

· End-point PCR analysis

The purified DNA was used as a template in an End-point PCR reaction for the Alu human polymorphism determination following the protocol of the Determination of the Alu polymorphism by PCR (DANAGEN-BIOTED, Spain).

· Real-Time PCR analysis

The purified DNA was used as a template in a Real-Time PCR reaction for the detection of Human Genomic DNA using the cfhDNA MONODOSE dtec-qPCR Kit (Genetic PCR SolutionsTM, Spain).

The target is a multiple-copy gene, 200 copies per genome, with a slow evolutionary rate.

Results and Discussion

· Detection of Human Genomic DNA

DNA quantity was assessed using both fluorescence (Qubit. Figure 3) and gel electrophoresis (Figure 4). The shown results are the average of three samples and all samples were eluted/hydrated in 50 µl Tris HCl 5 mM pH 8.5.

We can observe the DNA concentration of method 1 (salting-out) is double that method 2 (MicroSpin column) and method 3 (Robot).

The amount of DNA that can be obtained from saliva is variable between individuals, as seen with donor 1, that obtained a higher quantity of DNA.

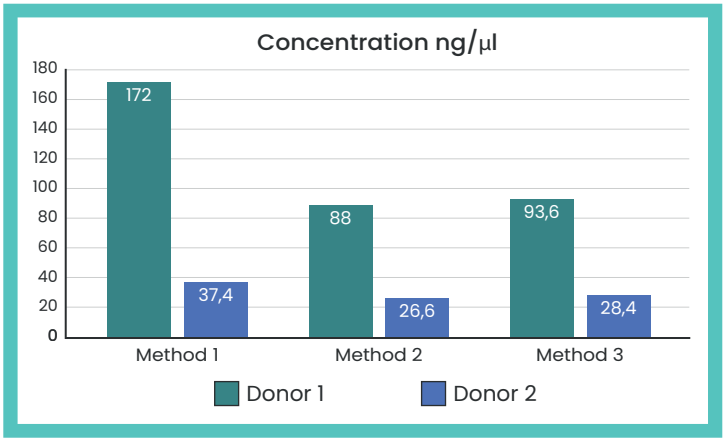
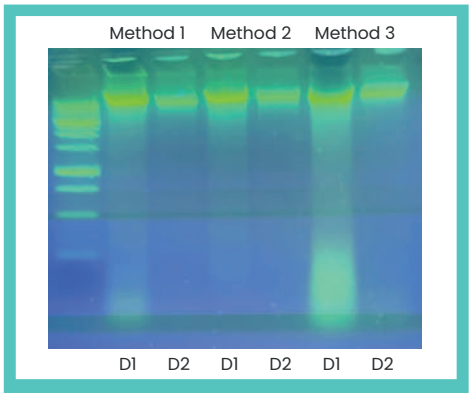


Figure 3. DNA concentrations of total DNA obtained by spectrophotometric analysis (Qubit)

The DNA obtained is high molecular weight (>23kb). The presence of large amounts of degraded RNA in donor 1 does not affect the quality of DNA but it may impact quantification methodologies (Nanodrop meazsures not shown). Smearing is seen in lanes (donor 1) where > 200 ng of DNA has been loaded, suggesting an overloading of the agarose gel rather than poor-quality DNA.

Figure 4. Gel electrophoresis of genomic DNA purified form preserved saliva samples



DNA quality was assessed by spectrophotometry (Nanodrop. Figure 5). All total nucleic acid isolated from all samples displayed A260/280 ratios > 1.75, demonstrating the high purity of the preparations. The A260/230 values are also good for method 2 (MicroSpin column) and lower than methods 1 and 3. The results are the average of three samples.

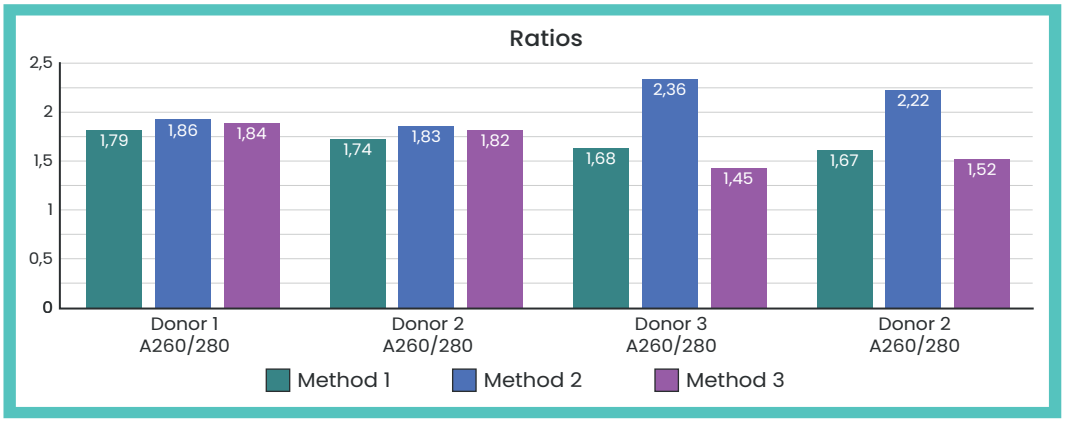


Figure 5. 260/280 and 260/230 ratios by spectrophotometric analysis (Nanodrop)

· Determination of Alu polymorphism by PCR

The Alu polymorphism studied here is an insertion found in intron 8 of the tissue plasminogen activator gene (TPA). This region is about 260-270 nucleotides long and the insertion is approximately 300 base pairs long, so the insertion will result in an increase of 570 base pairs.

It can be tested whether a person has an Alu insertion at the TPA locus by PCR amplification. If a person is homozygous for the insertion, agarose gel electrophoresis of the PCR product will produce a single band of 570 base pairs. If a person is heterozygous, having the insertion in one of the homologous chromosomes but not in the other, 2 bands will appear on the gel, one of 570 base pairs and another of 260 base pairs. If a person does not present this particular insertion on either of the homologous chromosomes, the PCR will result in a single 260 base pair band.

In Figure 6, the results of PCR amplification of the TPA locus obtained following the three different extraction methods are shown. All three methods produced good PCR results. We observed that donor 1 is heterozygous for the Alu insertion, presenting 2 bands (260 and 570 base pairs), while donor 2 is homozygous for the Alu insertion.

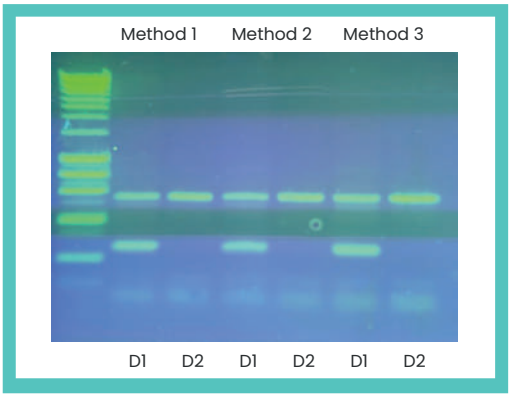


Figure 6. PCR analysis for the ALU insertion in intron 8 of the tissue plasminogen activator gene

· Real Time PCR assay for Total Human DNA

To verify that the extracted DNA was of high purity, we also performed a Real Time PCR assay.
To do so, we used the cfhDNA MONODOSE dtec-qPCR Kit (Genetic PCR Solutions TM, Spain). The target is a multiple-copy gene, 200 copies per genome, with a slow evolutionary rate.
Although we observed some variability in Cts, the amplification was successful with DNA from all three different methods (Fig.7).

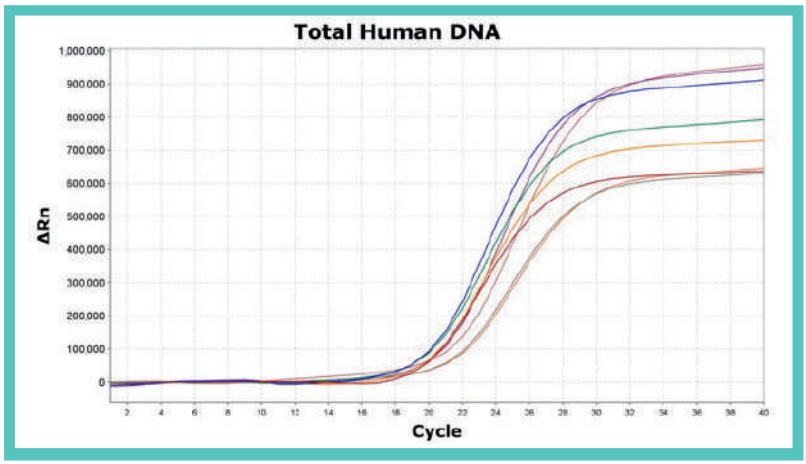


Figure 7. Amplification of Total Human DNA by qPCR analysis

Conclusion

Saliva collected in the DANASALIVA Sample Collection Kit is a non-invasive source of high quality of genomic DNA allowing a flexible extraction pipeline, regardless of the extraction method, manual or automatic, good results are obtained.

The DNA recovered from saliva is high molecular weight and performs identically to DNA isolated from blood for a broad range of common downstream applications, such as end-point PCR, qPCR, and further molecular detection methods.

DANASALIVA Sample Collection Kit

DANASALIVA Sample Collection Kit provides a safe and rapid all-in-one procedure for the collection, stabilization and transportation of **2 ml saliva samples at ambient temperature**.

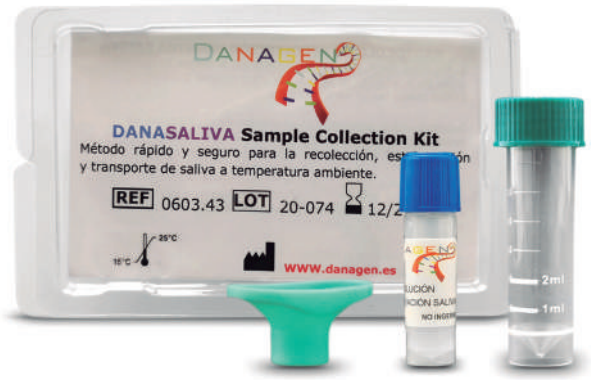
Our system effectively stabilizes buccal cell and white blood cells found in saliva without breaking them **over 1 year at room temperature**.

Saliva samples are collected by spitting inside the **collection funnel** which has been assembled with the **collection tube**. After collecting 2 ml saliva the contents of **saliva preservation solution** are then added and mixed with the collected saliva.

The saliva collection tube is sent to the laboratory for **DNA isolation and analysis using the DANAGENE SALIVA KIT or DANAGENE SPIN SALIVA DNA Kit**.

CE-IVD Marked version available:

- CE-IVDR marked in accordance with the European Commission Regulation (EU) No. 2017/746.
- Ideal for use in in vitro diagnostic workflows



DANASALIVA Sample Collection Kit RUO

REAL SALIVA DNA Sample CollectionKit CE-IVD

Specifications:

- Easy collection, transportation and processing.
- Painless, non-invasive collection.
- Samples can be mailed using the standard postal system.
- Compatible with most DNA isolation methods and can be automated.
- Sample remains stable for 1 year at room temperature, reducing transportation and storage costs.
- High quality DNA is suitable for sensitive downstream applications.

Ordering information:

Reference	Product Description	Preps
0603.43	DANASALIVA Sample Collection Kit	1
0603.43100	DANASALIVA Sample Collection Kit	100
0603.43500	DANASALIVA Sample Collection Kit	500
0603.41000	DANASALIVA Sample Collection Kit	1000
RBMSAL01DANCE	REAL SALIVA DNA SAMPLE COLLECTION CE-IVD	100

DANASWABS Sample Collection Kit

DANASWABS Sample Collection Kit provide a safe and rapid all-in-one procedure for the collection, stabilization and transportation of **saliva swab samples**. It contains a swabs + preservation buffer microtube.

We use Swabs specifically designed and dedicated to DNA collection, this swabs are certified DNase, RNase-Free and Human DNA-Free, as well as free of any PCR inhibitors. And tested in our laboratory as the highest yielding DNA.

After collection the swab is introduced into a **microtube containing a preservation solution**, thus the buccal cells can be transported and **stabilized for 1 year at room temperature and indefinitely at -20 or -80**.

The collection microtube is sent to the laboratory for DNA isolation and analysis using the **DANAGENE SWABS DNA KIT**.

Specifications:

- Unique swab matrix greatly improves DNA yields.
- Painless, non-invasive collection.
- Easy to handle and quick to use.
- Compatible with most DNA isolation methods and can be automated.
- Sample remains stable for 1 year at room temperature.
- High quality DNA is suitable for sensitive downstream applications.

Ordering information:

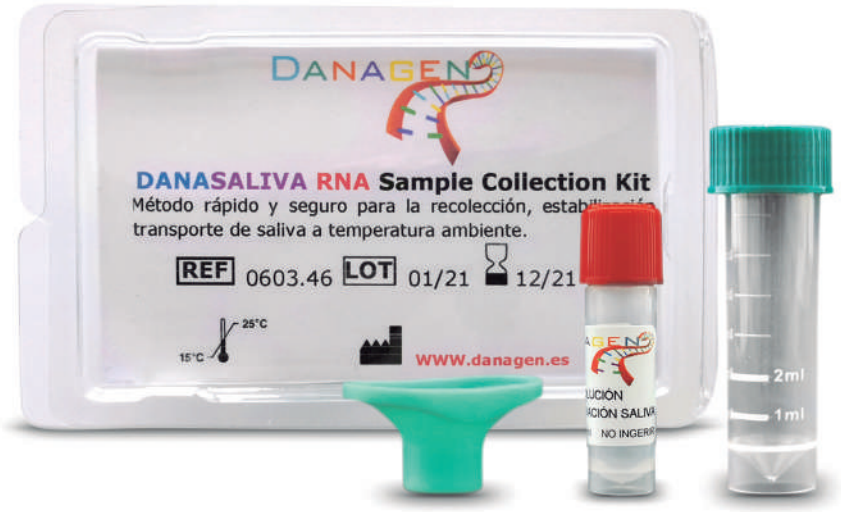
Reference	Product Description	Preps
0615.50	DANASWABS Sample Collection Kit	50
0615.100	DANASWABS Sample Collection Kit	100
0615.500	DANASWABS Sample Collection Kit	500
0615.1000	DANASWABS Sample Collection Kit	1000

DANASALIVA RNA Sample Collection Kit

Saliva, the most accessible and non invasive biofluid of our body, harbours a wide spectrum of biological analytes informative for clinical diagnostic applications. Recently, human RNA obtained from saliva was shown to be a biomarker for several diseases.

DANAGEN-BIOTED has developed a complete system that uses saliva as the sample source for expression studies.

1. DANASALIVA RNA Sample Collection Kit provides a safe and rapid all-in-one procedure for the collection, stabilization and transportation of **1 ml saliva samples at ambient temperature that stabilizes RNA from the moment of collection for 1 month**.



Specifications:

- Easy collection, transportation and processing.
- Sample volume: 1 ml saliva.
- Sample remains stable for 1 month at room temperature, reducing transportation and storage costs.
- Painless, non-invasive collection.
- Samples can be mailed using the standard postal system.
- Human mRNA expression profiling.

DANAGENE SALIVA RNA Kit

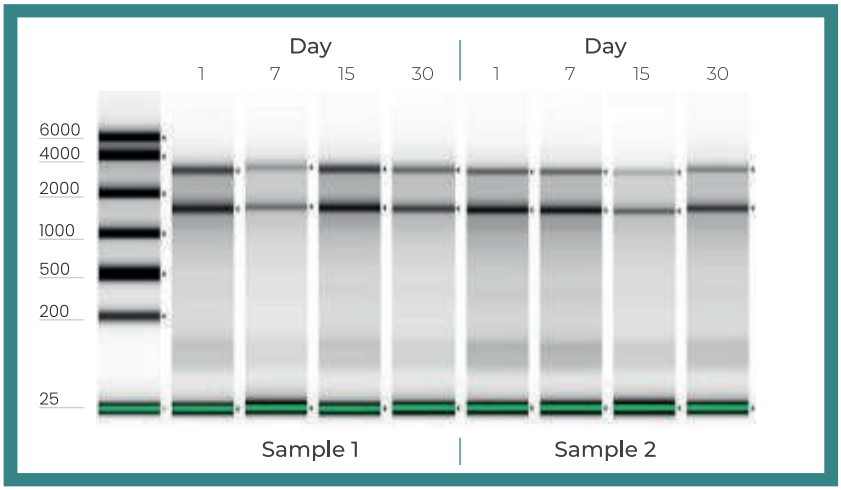
2. DANAGENE SALIVA RNA Kit has been designed for a fast and efficient purification of **total RNA from preserved saliva samples**.

The process includes a cell lysis with proteinase K followed by a precipitation of the proteins and part of genomic DNA. Later, by a precipitation with isopropanol, total RNA is obtained, which is finally rehydrated. Finally, for **removal of genomic DNA contamination** is used an approach consisting of two sequential filtrations with different Micro Spin columns.

Specifications:

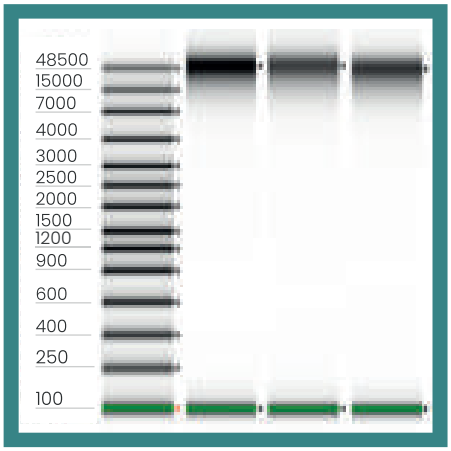
- Buffer-based RNA isolation combined with gDNA removal with columns.
- Sample volume: 600 µl of preserved saliva sample.
- Total RNA.
- A260/A280 Ratio: >1.8.
- Elution volume: 50 µl.

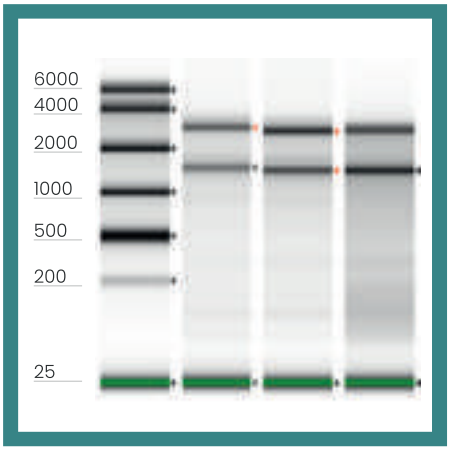
Application data:



RNA was isolated from aliquots of 600µl of preserved saliva samples with our **DANASALIVA RNA Sample Collection Kit**.

These samples were preserved at room temperature for 30 days. Isolation was carried out on day 1, day 7, day 15, and day 30 using our **DANAGENE SALIVA RNA Kit**. Quality was assessed using the Agilent 4150 TapeStation.





Parallel purification of DNA and RNA can be extracted into separated fractions using a modification of the **DANAGENE SALIVA RNA Kit** protocol. Quality was assessed using the Agilent 4150 TapeStation.

Ordering information:

Reference	Product Description	Preps
0603.46100	DANASALIVA RNA Sample Collection Kit	100
0603.46500	DANASALIVA RNA Sample Collection Kit	500
0603.461000	DANASALIVA RNA Sample Collection Kit	1000
0809.1	DANAGENE SALIVA RNA Kit	50

DANASTOOL Sample Collection MICROBIOME Kit

DANAGEN-BIOTED has developed a complete system for processing samples of human or animal feces. **DANASTOOL Sample Collection MICROBIOME Kit** is an integrated system for collection, transportation and storage of stool samples and subsequent DNA purification. Transportation of the stabilized DNA can be carried out in the DNA Stabilization solution without refrigeration at ambient temperature.

El DANASTOOL Sample Collection MICROBIOME Kit enables collection, storage and stabilization of stool samples. It comes in a tube with spoon and liquid stabilization solution that preserves the MICROBIOME profiling.

Specifications:

• Easy to use, designated for collection and safe transportation because the samples become Not infectious.

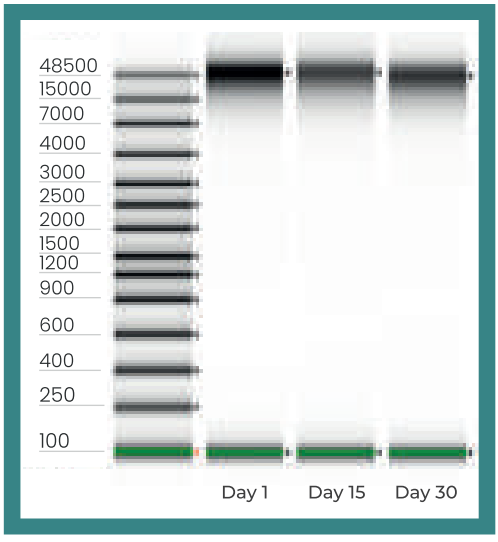
• It is not necessary to process the samples immediately.

• It stabilizes the DNA for several months at room temperature and at -20 or -80 indefinitely.

• Eliminate odour during processing.

• The DANASTOOL preservative solution and our DNA isolation method are capable of maintaining DNA integrity.

• Compatible with a variety of purification systems. The use of our DANAGENE MICROBIOME FECAL DNA Kit is highly recommended.



DNA was isolated from preserved stool samples with DANASTOOL Sample Collection Kit. These samples were preserved at room temperature for 1 month. Isolation was carried out on day 1, day 15, and day 30 using our DANAGENE MICROBIOME FECAL DNA Kit. Quality was assessed using the Agilent 4150 TapeStation

Ordering information:

Reference	Product Description	Preps
0617	DANASTOOL Sample Collection MICROBIOME Kit	50
0618	DANASTOOL Sample Collection MICROBIOME Kit	250

DANAGENE Protect Solution

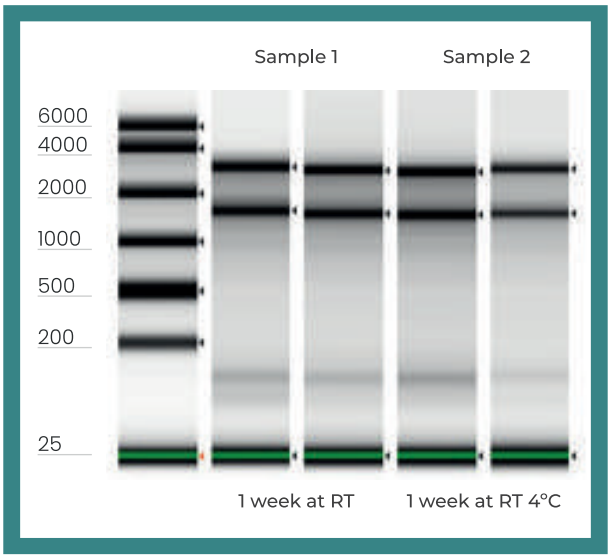
DANAGENE Protect Solution is a non- toxic solution that **allows the collection and storage of cells and tissues in different conditions and protect and stabilize the genomic DNA and RNA for its following isolation**.

DANAGENE Protect Solution is an aqueous and nontoxic tissue-holding liquid, which can in situ stabilize and protect RNA under non-frozen situation by rapid infiltrating fresh tissues and not affect RNA yield and integrity. Hence, RNA Stabilization Solution eliminates inconveniences to flash freeze samples in liquid nitrogen or take samples from different places. When fresh tissues immerged into RNA Stabilization Solution, RNA can be stored up to a day at 37° C, a week at 25° C, a month at 4° C and a long term at -20° C or -80° C.

Specifications:

- It removes the need of processing immediately the samples.
- The samples can be preserved for 2 weeks at room temperature (20-25°C); 1 month at 4°C and indefinitely at -20°C or -80°C.
- More flexibility as it simplifies the sample collection, it is not necessary to freeze samples in liquid N2 or in laboratory freezers.
- It is an alternative to the use of paraffin for protecting tissues.
- Allows the collection of samples in places that are out from the laboratories.
- Alt is compatible with the DANAGENE purifications kits.

Application data:



Parallel purification of DNA and RNA RNA was isolated from Sf9 cells preserved saliva samples with DANAGENE Protect Solution. These samples were preserved at room temperature for 7 days and 1 month at 4°C. Isolation was using our **DANAGENE TISSUE/CELLS RNA Kit**. Quality was assessed using the Agilent 4150 TapeStation.

Ordering information:

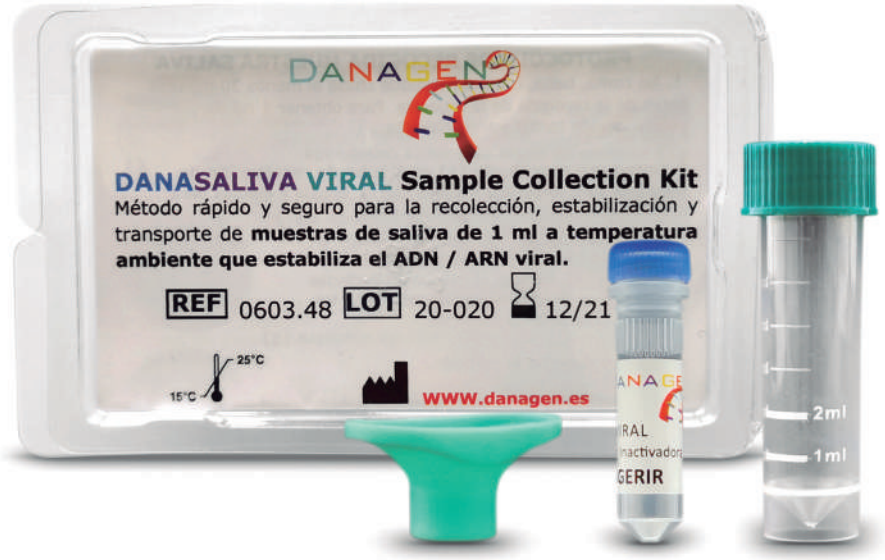
Reference	Product Description	Preps
DPT100	DANAGENE PROTECT SOLUTION	100 ml
DPT500	DANAGENE PROTECT SOLUTION	500 ml

DANASALIVA VIRAL Sample Collection Kit

DANASALIVA VIRAL Sample Collection Kit provides a safe and rapid all-in-one procedure for the collection, stabilization and transportation of **1 ml saliva samples at ambient temperature that stabilizes viral DNA/RNA**.

DANAVIRAL Stabilization Solution effectively inactive viruses and prevent nucleic acid degradation in samples collected with our system, **resulting non-infectious samples to be handled and shipped safely**.

Sample is preserved at ambient temperature (**DNA>1 year; RNA up to 1 month**). Samples can be frozen (-20/-80°C) for prolonged periods.

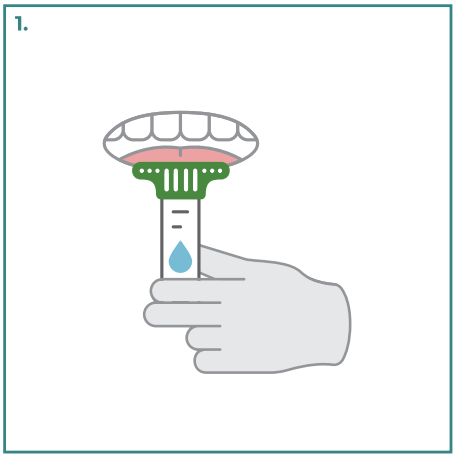


Specifications:

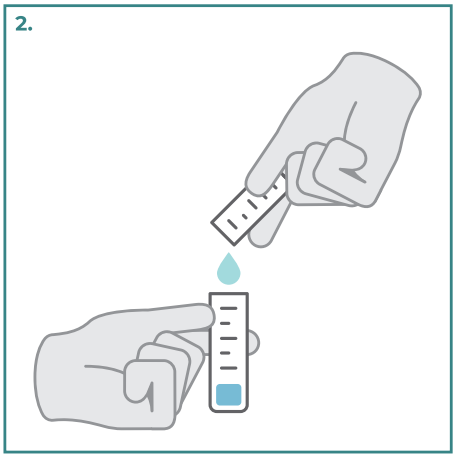
- Used for collection, storage and transportation of viral samples from 1 ml saliva.
- Inactivate microorganisms and viruses for safe and easy transport and handling.
- It can be used for isolating the Human Genomic DNA and viral DNA/RNA.
- Room temperature transport.
- Preserve total DNA, including viral DNA, at room temperature (4°-25°C) 1 year.
- Preserve total RNA, including viral RNA, at room temperature (4°-25°C) 1 month.
- Compatible with most DNA and RNA isolation methods.

We recommend to use our DANAGENE Kits for DNA/RNA viral isolation

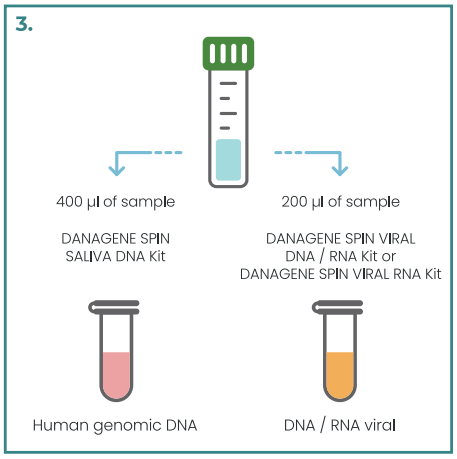
Product workflow overview:



Collect 1 ml of saliva



Add 1 ml of DANAVIRAL Stabilization Solution



Purification of Human Genomic DNA or DNA / RNA Viral

Kit components:

	100 Preps	Stock
DANAVIRAL Stabilization Solution	2ml x 100 units	Room temperature
Funnels	100 units	Room temperature
Collection tubes	100 units	Room temperature

Ordering information:

Reference	Product Description	Preps
0603.48	DANASALIVA VIRAL Sample Collection Kit	1
0603.48100	DANASALIVA VIRAL Sample Collection Kit	100

DANASWAB VIRAL Sample Collection Kit

DANASWAB VIRAL Sample Collection Kit is designed **for collection, ambient storage and transport viral DNA/RNA from samples collected using a swab**, including nasal, throat, saliva, fecal, surfaces and wherever a swab may be deemed appropriate per application.

DANAVIRAL Stabilization Solution effectively inactive viruses and prevent nucleic acid degradation in samples collected with the provided swab, resulting non-infectious samples to be handled and shipped safely.

· NASAL SWAB

· BUCCAL SWAB

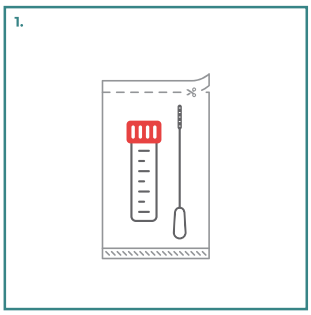
· DUO (NASAL SWAB + BUCCAL SWAB)

Specifications:

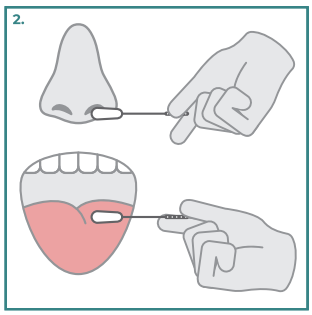
- Used for collection, storage and transportation of viral samples.
- Inactivate microorganisms and viruses for safe and easy transport and handling.
- Compatible with many sample types, including nose, mouth, throat, biological fluids, environmental samples.
- Room temperature transport.
- Preserve total DNA, including viral DNA, at room temperature (4º-25ºC) 1 year.
- Preserve total RNA, including viral RNA, at room temperature (4º-25ºC) 1 month.
- Compatible with most DNA and RNA isolation methods.

We recommend to use our DANAGENE Kits for DNA/RNA viral isolation

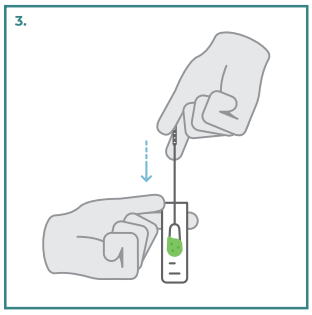
Product workflow overview:



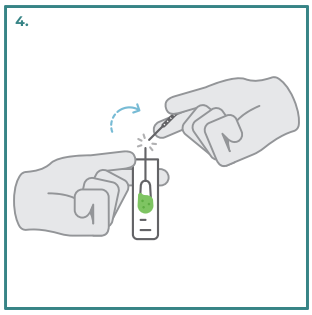
Open package containing swab and collection tube. Remove the individually wrapped swab and the preservative tube. **Do not touch the swab tip**.



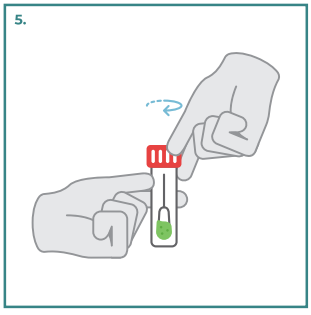
Swab the sample. For fecal and soil samples collect approximately no more than 100 µg.



Open the collection tube and insert swab tip into the microtube.



Break the swab tip (breaking point 20-30 mm) leaving the swab tip in the collection tube. **Make sure that the swab is in contact with the solution**.



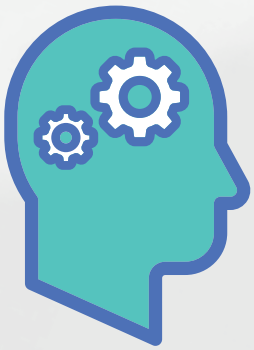
Replace the tube cap, securing tightly and invert microtube several times. The sample is stabilized and ready for transport/storage prior to purification of DNA and/or RNA.

Ordering information:

Reference	Product Description	Preps
0603.47 NASO	DANASWAB VIRAL Sample Collection Kit	100
0603.47 ORO	DANASWAB VIRAL Sample Collection Kit	100
0603.47 DUO	DANASWAB VIRAL Sample Collection Kit	100

DNA / RNA Purification

If you want, we can isolate or purify your DNA / RNA from any biological sample.



Customized Sevices

We can work in collaboration with you or independently to customize our protocols and procedures to adapt them in the best way to your needs. Our extraction and purification products are not closed products and we can change the standard size of the kit and the different components to adapt it to your needs.

DNA / RNA Purification

You can send us your sample and we can study previously the ideal kit and protocol for the type and size of your sample.

OEM Services

We can offer our products in OEM or bulk.

Life Science Research

Life Science Research

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