DANAGENE MICROBIOME VAGINAL DNA KIT

Ref.0624 50 Preps

1. 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 vaginal microbiome harbors diverse communities of microorganisms, known as vaginal flora which has an important impact on women's health as well as that of their newborns

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*[1]. 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 microbiota depends on age, menstruations, hormonal fluctuations, sexual behaviors, 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.

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

Features:

VAGINAL SELF-COLLECTION SWAB

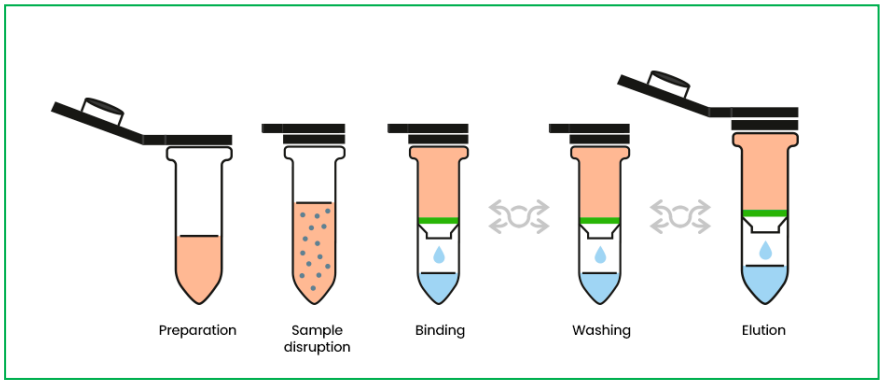
The new solution for home self-sampling, collection, shipping and easy processing in the laboratory

"Women are really busy" the self-collected vaginal swab is a newer method of collection that offers several advantages compared to traditional methods:

- Reduce waste of time for women that don't want to take time off from work
- Absolute privacy
- Increased comfort
- Reduced anxiety

DANAGENE MICROBIOME VAGINAL DNA Kit

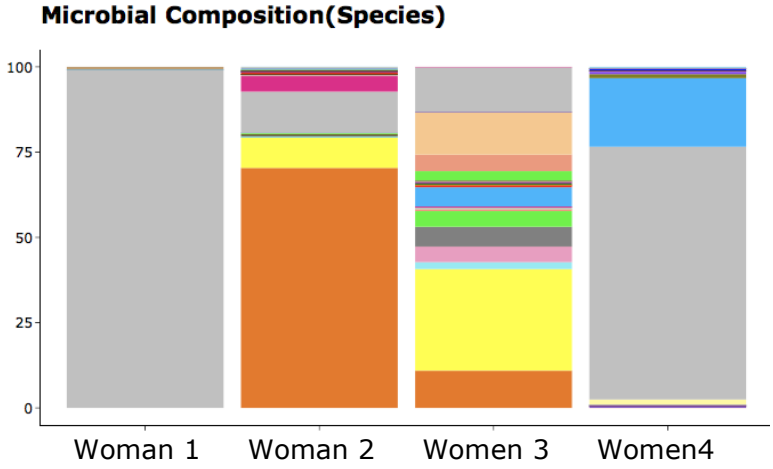
• Designed for rapid purification of highly pure microbial DNA for microbiome analysis.
• Silica-membrane technology with MiniSpin columns.
• Bead Microtubes for efficient lysis included in combination liquid Proteinase K.
• Sample material: vaginal swabs samples.
• Typical yield: it depends on patient.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



Beginning with a bead-beating protocol, cells are lysed through a combination of mechanical force, heat and detergent, vortexed using horizontal adapter for the Vortex Genie 2 Vortex or using others common disruption devices.

Appropriate DNA binding conditions to the Microbial DNA Columns are achieved by addition of large amounts of chaotropic salts (Binding Buffer) to the lysate. Contaminants are removed by two efficient washing steps. Afterwards, The resulting DNA is recovered in a DNA-free Tris buffer to use for subsequent reactions.

Microbial composition of vaginal sample preserved at room temperature for 4 different women with VAGINAL SELF-COLLECTION SWAB . Vaginal samples were taken using our system and stored at room temperature. They were processed with the DANAGENE MICROBIOME VAGINAL DNA Kit. The extracted DNA was subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Fig.1



2. KIT COMPONENTS

VAGINAL SELF-COLLECTION SWAB 50 Units

ISOLATION KIT	50 Preps	Storage
CTAB Extraction Buffer	**50 ml**	Room Temperature
Binding Buffer	**15 ml**	Room Temperature
Desinhibition Buffer*	**18 ml**	Room Temperature
Wash Buffer *	**10 ml**	Temperatura ambiente
Elution Buffer	**10 ml**	Temperatura ambiente
Bead Microtubes	**50 units**	Temperatura ambiente
Proteinase K*	**30 mg**	-20ºC
Microbial DNA Columns	**50 units**	Temperatura ambiente
Collection Tubes	**100 units**	Temperatura ambiente

(*) These solutions must be prepared as indicated in the Preliminary Preparations section of the protocol.

Equipment and additional reagents required

- Microcentrifuge.
- Microcentrifuge tubes, DNase-free, 1.5 mL and 2.0 ml
- Ethanol 100%.
- Heat block, dry bath, or water bath (70°C).
- For vortex bead homogenization: hands-free adapter for vortex mixer, with horizontal tube orientation, we recommend the Vortex Genie 2.
- (Optional) alternative to vortex bead homogenization: Bead mill homogenizer.

3. PROTOCOL

3.1 Preliminary Preparations

- Dissolve the proteinase K in **1.3 ml of nuclease-free water** and store at –20ºC. It is recommended to do several aliquots to avoid many thaw/freeze cycles. At this temperature it is stable for 1 year.

- **Add 10 ml of Ethanol 100 %** to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation.

- **Add 40 ml of Ethanol 100 %** to the Wash Buffer. Keep the container closed to avoid the ethanol evaporation.

- Pre-heat the Elution Buffer at 70ºC.

3.2 General Remarks

- Take vaginal samples following the instructions. Our swabs has the ability to preserve **nucleic acids stability up to 4 weeks at room temperature**

- The procedure is optimized by using "beads" in a vortex with horizontal agitation (Vortex Genie 2 or similar). Make sure that the vortex adapter allows horizontal agitation.

- Adapters with a vertical tube orientation my not agitate properly.

- You can use "Bead mil" homogenizers such as FastPrep, Precellys and others but following the manufacturer's instructions to optimize the lysis of the sample. IMPORTANT: Many modern disruption devices can cause very high energy input in bead tubes. Depending on bead tube type and content (beads, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause breaking up of the bead tubes! **It is the responsibility of the user to perform initial stability test for the used bead tubes under the conditions used!** Perform initial test with water instead of lysis buffer and moderate machine setting (low frequency, short time) in order to avoid spillage of chaotropic lysis buffer in case of tube breakage.

- In addition to the standard method, several modifications are possible to increase yield, concentration, and convenience.
  Convenient elution (standard elution): For convenience, elution can be performed by one time addition of 100 µL elution buffer onto the column.
  High yield: Two serial elutions of 100 µL each for total elution volume of 200 µL.
  High concentration: Use initial 100 µL eluate for second elution – 100 µL total elution volume, 2 elutions.

3.3 Protocol for microbial DNA isolation from vaginal samples

1. Add **900 µl of CTAB Extraction Buffer** to 2.0 ml microtube.

Cutting the swab head and some of the handle into the microtube, so the microtube can be closed. Vortex vigorously to release cells from the brush

2. **Add 25 µl of Proteinase K. Incubate at 70ºC for 10 minutes.**

3. Remove the swab head of the lysis solution, rubbing against the walls to collect the maximum amount of liquid. **Transfer 900 µl of lysate to a "Bead microtube".**

4. **Homogenize** by bead beating for 10 minutes at maximum speed on the Vortex Genie 2 or similar using a **horizontal adapter.**

5. **Centrifuge at 14.000 rpm for5 minutes**.

6. Transfer up to 500 µL of the supernatant to a clean microcentrifuge tube.

7. Add **250 µl of Binding Buffer** and vortex briefly.

8. Load the sample mixture onto a **Microbial DNA columns-tube assembly**, and Discard the flow-through.
   Ensure that the entire sample mixture has passed into the collection tube by inspecting the column. If sample remains in the column, centrifuge again at 14.000 rpm for 1 minute.

8. Place the Microbial DNA column in a clean collection tube, add **500 µl of Desinhibition Buffer.Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

9. Add **700 µl of Wash Buffer. Centrifuge at 14.000 rpm for 1 minute.**

10. **Dry silica membrane.** Centrifuge at 14.000 rpm for 3 minutes.

11. Place the Microbial DNA Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Pre-heat the Elution Buffer** at 70ºC. Incubate **at room temperature** for **2 minutes**.

12. **Centrifuge** the spin column-tube assembly **at 14.000 rpm for 1 minute**, The purified DNA is in the microtube.

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es