DANAGENE MICROBIOME RNA KIT

Ref.0622 50 Preps

1. INTRODUCTION

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 (e.g. feces, soil, biological fluids and others swab samples).

In this procedure, the microorganisms are efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads.

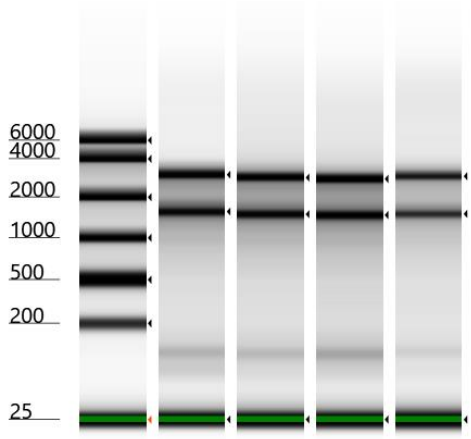
Features:

- **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.**
- **No phenol/chloroform extraction or ethanol precipitation is necessary.**

Applications:

- **Next-Generation Sequencing.**
- **RT/qPCR.**
- **Pathogen typing.**

High-Quality RNA



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

2. COMPONENTS KIT

	50 preps	Storage
RNA Lysis Buffer	**32 ml**	Room temperature
Wash Buffer *	**10 ml**	Room temperature
Nuclease-Free water	**8 ml**	Room temperature
Proteinase K *	**30 mg**	-20ºC
gDNA Removal Column	**50 units**	Room temperature
RNA Columns	**50 units**	Room temperature
Collection Tubes	**100 units**	Room temperature
Bead Microtubes	**50 units**	Room temperature

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

PRECAUTIONS: The RNA Lysis Buffer contains guanidium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined bleach.

1.1 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 (65°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 40 ml of Ethanol 100 %** to the Wash Buffer. Keep the container closed to avoid the ethanol evaporation.

3.2 General Remarks

- 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 mill" 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.

3.3 Protocol for RNA extraction from samples preserved in DANASWAB Sample Collection MICROBIOME Kit

IMPORTANT: Prior to purification of RNA wait at least 24 hours and vortex vigorously to properly homogenize the preserved sample.

1. Add **200 µL of MICROBIOME Stabilization Solution with preserved swab sample** in a Bead microtube **+ 600 µl of RNA Lysis Buffer + 25 µl of Proteinase K. Incubate at 70ºC for 10 minutes.** Shake tubes manually several times during incubation.
2. **Homogenize** the sample by bead beating for **10 minutes** at maximum speed on the Vortex Genie 2 or similar using a **horizontal adapter.**
3. **Centrifuge at 14.000 rpm for 5 minutes**. Transfer up to **600 µL of the supernatant to the gDNA Removal Column** and centrifuge for 1 minute at 8.000 rpm.
4. Discard the column and **continue with the flow-through and add 600 µl of Ethanol 100%.** Mix well.

5. Take an **RNA column** plus its collection tube and add the mixture from point 4. **Centrifuge at 8,000 -10,000 rpm for 30 seconds**. Pass the sample in 2 times as the volume exceeds the capacity of the column.

6. Add **200 µl of Ethanol 100%. Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

7. Add **700 µl of Wash Buffer. Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

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

9. Place the RNA Column into a 1.5 mL nuclease-free tube (not provided) and add **50-100 µL Nuclease-Free water.** Incubate **at room temperature** for **2 minutes**.

10. **Centrifuge** the spin column-tube assembly **at 14.000 rpm for 1 minute**, then discard the column. The purified RNA is in the tube.

4. PROBLEM GUIDE AND POSSIBLE ANSWER

Due to the great environmental samples variety that can be treated, it becomes difficult to generalize possible problems and answers. For this reason, we recommend to contact **DANAGEN-BIOTED** Laboratory Technical Service for any question regarding the protocols, specific soil samples or any problem you may have during the process.
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