REAL MICROBIOME FECAL DNA KIT

Ref. RBMEGS16 50 PREPS

1. INTRODUCTION

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

a) **up to 200 mg fresh and frozen human or animal stool samples.**
b) **Stool homogenate from 0.50-1.0 gr stool and stabilized in 8 ml Real STOOL Sample Collection Kit.**

In this procedure, the microorganisms are efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads. Inhibitors are eliminated by precipitation using a propietary cleanup buffer. The sample is then applied to a microspin column and the DNA that is bound to the column undergoes a single wash step before elution.

Features:

- **Designed for a fast and easy purification microbial DNA from different types of stool samples.**
- **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.**
- **No phenol/cloroform extraction or ethanol precipitation is necessary.**

Aplications:

- **Microbiome analysis**
- **PCR applications.**
- **RFLP analysis.**
- **Patogehn typing.**
- **Mutation analysis.**

2. COMPONENTS KIT

	50 preps	Storage
CTAB Extraction Buffer	**50 ml**	Room temperature
EC Buffer	**10 ml**	Room temperature
Binding Buffer	**15 ml**	Room temperature
Desinhibition Buffer*	**18 ml**	Room temperature
Wash Buffer *	**10 ml**	Room temperature
Elution Buffer	**10 ml**	Room temperature
Bead Microtubrs	**50 unidades**	Room temperature
Proteinase K*	**30 mg**	-20ºC
Microbial DNA Columns	**50 unidades**	Room temperature
Collection Tubes	**100 unidades**	Room temperature

(*) 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

Sample size

Depending on the size of the stool sample, mix the sample completely to generate a homogeneous sample before weighing and transfer a sample (200 mg) to the microtube with the beads.

The kit is optimized for the processing of 200 mg of human feces. For animal samples it is recommended to reduce the amount of sample (80-100 mg), this can lead to better results.

Very dry samples (mouse, rabbit, etc.) can absorb the CTAB Extraction Buffer, producing an insufficient amount of sample after the first centrifugation step. In these cases, it is recommended to reduce the sample size (60-80 mg) and increase the volume of the CTAB Extraction Buffer.

When not working with standard stool samples it is recommended to evaluate the initial amount of sample needed, as well as the CTAB Extraction Buffer to be used to obtain 600 µl of lysate in the first centrifugation step (step 4 of the protocol).

The human stool samples may contain undigested food material, these particles should not be transferred to the microtube with the beads.

Sample lysis

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.

3.3 Protocol for DNA extraction from 200 mg of fresh or dry stool

NOTE: It is recommended to begin with big quantities of stool samples when the DNA is not distributed homogeneously or it is in small quantities in the sample. Samples of smaller size can be processed to the suitable overalls when it is required to eliminate to the maximum the possible inhibitors of the PCR.

In general, for good results of PCR to use the minimum quantity possible of DNA, the volume will never exceed of 10% of the final volume of the mixture of PCR. It is recommended to add BSA to a final concentration of 0.1 □g / □l of the mixture of PCR and to use HOT Star polymerase.

1. Wheigh **50-200 mg of stool sample** and place them in 2.0 ml **bead microtube**. Add **1.0 ml de Tampón de Extracción CTAB**.

2. Resuspend the sample with simple shaking of the microtube or micropipette. No vortex. **Incubate at 70 ° C for 10 minutes.**

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

4. **Centrifuge at 14.000 rpm for 5 minutes**. Transfer up to **600 µL of the supernatant** to a clean microcentrifuge tube.
   IMPORTANT: A layer of debris may be present on top of the bead pellet. Avoid transfer of this debris with the supernatant.

5. Añadir **250 µl EC Buffer Vortex.** Incubate at 4ºC for 5 minutes

6. **Centrifuge at 14.000 rpm for 5 minutes**. A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up **500 µl of supernatant** that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 1.5 ml microtube.

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

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

9. Add the lysate into reservoir of a combined Microbial DNA Column–collection tube assembly. **Centrifuge at 10.000 rpm for 60 seconds.** Remove the collection tube.

10. Place the Microbial DNA column in a clean collection tube, add **500 µl of Desinhibition Buffer.Centrifuge at 12.000 rpm for 1 minute.** Discard the flow-through.

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

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

13. Place the Microbial DNA Column into a 1.5 mL nuclease-free tube (not provided) and add **100-200 µL Pre-heat the Elution Buffer** at 70ºC. Incubate **at room temperature** for **2 minutes**.

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

3.4 Protocol for DNA extraction from stool samples STABILIZED into Real STOOL Sample Collection Kit

SAMPLE COLLECTION:

1. Using the spoon attached to the lid take stool sample from 2-3 different sites and transfer the samples to the preservative fluid. Approximately half a spoon per take is enough. The liquid allows to stabilize 0.5-1.0 gr of sample. **It is recommended:** Depending on the size and characteristics of the stool sample, mixing the sample completely to generate a homogeneous sample in this way will not be necessary to take sample from 3 different sites of the sample.
2. Close the container well and shake to achieve the homogenization of the fecal matter with the preservative liquid. This will be achieved more quickly depending on the consistency of the fecal matter, for hard consistencies this can be achieved by helping with the spoon, or stirring the tubes every day until the day of extraction.
3. Label the stool tube with your full name and date of collection.
4. Send the sample to the laboratory, sending it to room temperature. The sample is stable for several months at room temperature (15-25ºC) and indefinitely at -20 o -80ºC.
5. For extraction, several different methods can be used, **the use of our Real MICROBIOME FECAL DNA Kit is recommended.**

MICROBIAL DNA EXTRACTION:

1. Transfer **1.0 ml of stabilized stool sample** to a 2.0 ml **bead microtube** containing particles.

   Before transferring the sample, make sure that the sample is completely homogenized, for the transfer it is recommended to cut a 1000 µl tip to make the mouth wider and take 2 x 500 µl, mixing the sample well with the micropipette.

2. Resuspend the sample with simple shaking of the microtube or micropipette. No vortex. **Incubate at 70 ° C for 10 minutes.**

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

4. **Centrifuge at 14.000 rpm for 5 minutes**. Transfer up to **600 µL of the supernatant** to a clean microcentrifuge tube.

IMPORTANT: A layer of debris may be present on top of the bead pellet. Avoid transfer of this debris with the supernatant.

5. Add **250 µl EC Buffer Vortex.** Incubate at 4ºC for 5 minutes

6. **Centrifuge at 14.000 rpm for 5 minutes**. A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up **500 µl of supernatant** that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 1.5 ml microtube.

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

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

9. Add the lysate into reservoir of a combined Microbial DNA Column–collection tube assembly. **Centrifuge at 10.000 rpm for 60 seconds.** Remove the collection tube.

10. Place the Microbial DNA column in a clean collection tube, add **500 µl of Desinhibition Buffer.Centrifuge at 12.000 rpm for 1 minute.** Discard the flow-through.

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

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

13. Place the Microbial DNA Column into a 1.5 mL nuclease-free tube (not provided) and add **200 µL Pre-heat the Elution Buffer** at 70ºC. Incubate **at room temperature** for **2 minutes**.

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

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of Durviz S.L. durviz@durviz.com.