REAL MICROBIOMAL DNA KIT

Ref.RBMEGS17 50 Preps

1. INTRODUCTION

Real 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 Real 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.

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.

Features:

- Designed for rapid purification of **highly pure genomic DNA from microorganisms**(gram-negative and gram-positive bacteria, yeast and fungi).
- Suitable for a large variety of starting materials: **Microbial cultures and agar plates.**

- **Bead Microtubes** for efficient lysis inluded in combination liquid **Proteinase K.**

Applications:

- Total DNA from mcrobial cultures.

- Typical downstream applications: PVR, real-time PCR, southern blotting, enzymatic reactions.

2. KIT COMPONENTS

	50 Preps	Storage
CTAB Extraction Buffer	**45 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 Microtubes	**50 units**	Room Temperature
Proteinase K*	**30 mg**	-20ºC
Microbial DNA Columns	**50 units**	Room Temperature
Collection Tubes	**100 units**	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

- Cells should be harvested from fresh microbial cultures by sedimentation via centrifugation. Supernatant should be removed by aspiration. Microbial cell pellets can be used fresh or stored at -20 °C to -80 °C before starting DNA isolation.

- 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 microbial cultures or agar plates

Microbial cultures: Harvest cells from a culture by centrifugation in a microcentrifuge tube (not provided). Discard supernatant. Add **800 µl of CTAB Extraction Buffer**. Resuspend the pellet using a micropipette. No vortex.

Up to approximately 50 mg of wet weight microbial cell culture pellet can be used as sample material.

Agar plates: Using a bacterial sloop transfer up 50 mg of colonies directly into the beads microtube containing the **800 µl CTAB Extraction Buffer**. Be careful not to take agar. Resuspend the pellet using a micropipette. No vortex.

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

2. **Homogenize** 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**.

4. Transfer up to 500 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 of Binding Buffer** and vortex briefly.

6. 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.

7. 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.

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

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

10. 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**.

11. **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