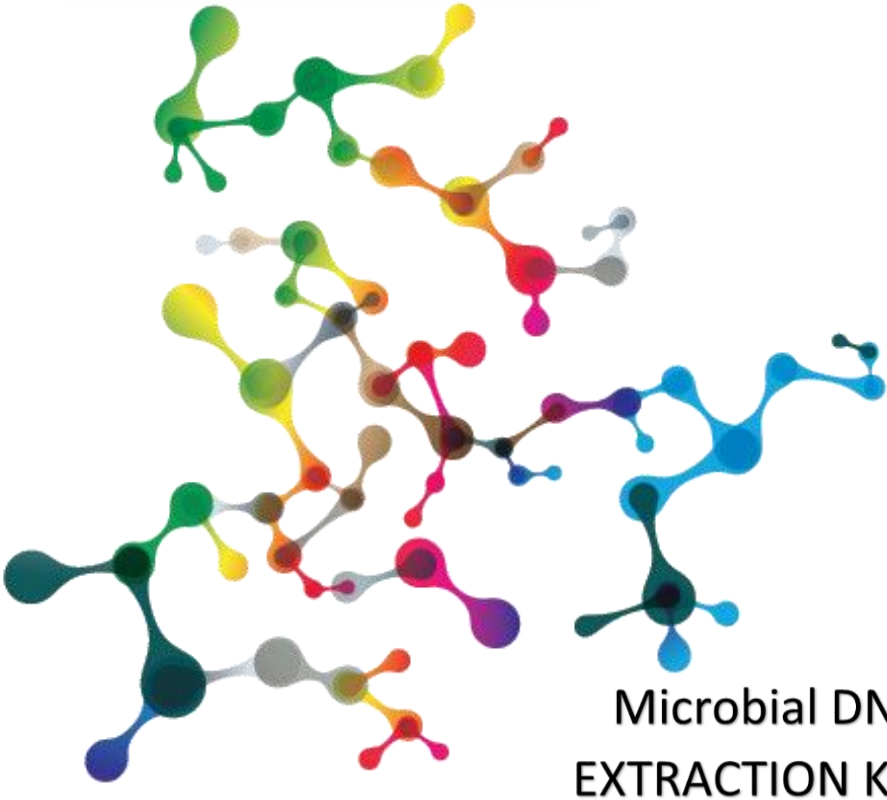
BIOPREMIER



Microbial DNA EXTRACTION KIT – Bead beating

BIOPEXT-0619

CONTACT

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BPMR is certified ISO 9001:2015

INTRODUCTION AND PRODUCT DESCRIPTION

The Biopremier Microbial DNA extraction Kit – Bead beating is designed for a fast and efficient purification of microbial DNA (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 Biopremier Microbial DNA extraction Kit – Bead beating replaces enzymatic lysis by using mechanical disruption of cell wall structures with specialized beads. 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. 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.

Applications:

- **For rapid purification of high-quality and ready-to-use genomic DNA from microrganisms (gram-negative and gram-positive bacteria, yeast and fungi).**
- **Total DNA from microbial cultures**

Features:

- **Bead Microtubes for efficient lysis included in combination liquid Proteinase K.**
- **No organic extraction or alcohol precipitation.**
- **Complete removal of contaminants and inhibitors for reliable downstream applications.**
- **Typical yield: 5 - 25 µg genomic DNA.**
- **Elution volume: 100 µL.**
- **High quality DNA obtained that can be directly used in PCR, Southern, any enzymatic reaction, cloning, etc.**

CONTENTS AND STORAGE

Name Tube	Volume or Units	Storage
	50 preps	
Lysis buffer	45 mL	RT
Binding buffer	15 mL	RT
Proteinase K *	30 mg	- 20ºC
Wash buffer 1 **	18 mL	RT
Wash buffer 2 **	10 mL	RT
Elution buffer	10 mL	RT
Bead Microtubes	50 units	RT
Microspin columns	50 units	RT
Collection Tubes	100 units	RT

If properly stored, see the expiration date for the stability of the Kit. RT: room temperature.

Notes

* Reconstitute Proteinase K by adding nuclease-free water (Molecular Biology grade) as indicated on vial(s) and stored at -20ºC. It is recommended to do several aliquots to avoid thaw/freeze cycles. At this temperature is stable for 1 year.

** Add ethanol (96-100%) to Wash buffers prior to use as indicated on the bottle(s). Keep the containers closed to avoid the ethanol evaporation.

MATERIAL REQUIRED BUT NOT SUPPLIED

Microcentrifuge tube (1.5 – 2.0 mL; 15 – 50 mL)
Micropipettes and micropipette filter tips (10 – 100 µL and 100 – 1000 µL)
Vortex
Microcentrifuge, able to operate up to 15.000 g.
Heater block (preferably) or Water bath
Powder-free gloves
Ethanol 100 %
For vortex bead homogenization: hands-free adapter for vortex mixer, with horizontal tube orientation

WARNINGS AND PRECAUTIONS

These products are exclusively for in vitro use
The test requires qualified staff to prevent the risk of erroneous results
Do not mix reagents from different batches

Do not use reagents from other manufacturer's products

Wear disposable gloves, laboratory coats when handling specimens and reagents.

Use sterile pipette tips with filters

Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately

Both the Lysis, Bindind buffers and Washing buffer 1 contain guanidine hydrochloride, which can form reactive components when combined with bleach (sodium hypochlorite). Also it is a irritant agent, for this reason we recommend to use gloves and glasses for its manipulation

Material Safety Data Sheets (MSDS) are available on request

Waste must be treated and disposed of in compliance with the appropriate safety standards

Clean periodically the working space with at least 5% of sodium hypochlorite

It is strongly recommended to have dedicated areas, materials, and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures

KIT USAGE INFORMATION

The kit contents should be mixed slightly before use.

Under cool environmental conditions, a precipitate may form in the Lysis buffer. In this case, the component should be heated to dissolve precipitate approximately 5minutes at 37^{o}C and thoroughly shaken prior use.

SAMPLE LYSIS

Processing time will vary based on sample input and bead beater. Times may be as little as 5 minutes when using high-speed cell disrupters (FastPrep) or as long 10 - 20 minutes when using lower speeds (Disruptor Genie). The procedure was optimized in a vortex with horizontal agitation (Vortex Genie 2 or similar). Make sure that the vortex adapter allows horizontal agitation; This is a cost-effective method for recovery of high-quality microbial DNA. Adapters with a vertical tube orientation my not agitate properly.

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.

PROCEDURE

PRELIMINARY PREPARATIONS

- ⚠ Make sure that Proteinase K and Wash Buffers were prepared according to "*Contents and Storage*" section, page 2
- ⚠ Preheat Lysis buffer and Elution buffer to 70ºC

1. GENOMIC DNA ISOLATION

Concentration step

1. Harvest cells from a culture by centrifugation in a microcentrifuge tube (not provided). Centrifuge at >**10.000 g for 10 minutes**. Remove the supernatant.

   It is recommended up to 50 mg of wet weight microbial culture pellet

Lyse cells step

2. Add **800 µL of Lysis Buffer.** Resuspend the pellet using a micropipette. No vortex. Transfer to a 2 mL bead microtube containing particles.

3. Add **25 µL of Proteinase K** and incubate for **10 minutes at 70ºC**

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 >**10.000 g for 5 minutes**

DNA binding step

6. Transfer up to **500 µL of the supernatant** to a clean microcentrifuge tube and add **250 µL of Binding Buffer.** Vortex briefly

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

7. Place the spin column in a 2 mL collection tube

8. Transfer **600 µL of the mix** to the column, and centrifuge at >**10.000 g for 1minute.** Discard flow-through

9. Repeat step 8 to **load the remaining sample**

Washing step

10. Place the Spin column in a new collection tube and add **500 µl of Wash Buffer 1** to the reservoir

11. Centrifuge at >**10.000 g for 1 minute.** Discard flow-through

12. Add **700 µl of Wash Buffer 2** to the reservoir and centrifuge at >**10.000 g for 1 minute**. Discard flow-through

13. Centrifuge at >**10.000 g for 3 minutes** to remove the residual ethanol

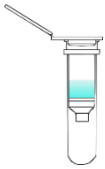
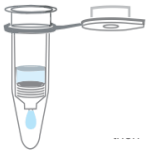
Elution step

14. Transfer the Spin column to a new 1.5 ml microcentrifuge tube and pipette **100 µL of Elution Buffer** (preheated at 70ºC) onto the membrane. Incubate at room temperature for 2 minutes

    Decrease the volume of elution buffer if a higher DNA concentration is desired.

15. Centrifuge at >**10.000 g for 1 minute**. Discard the spin column and use DNA immediately or store at -20ºC

WORKFLOW (1. ENRICHMENT CULTURE)

		PROCEDURE 1 and 3
1 CONCENTRATION STEP		Centrifuge the microbial culture >10.000 x g, 10 min
2 LYSE CELLS		800 µL Lysis buffer transfer to a bead microtube + 25 µL Proteinase K; 70ºC, 10 min homogenize, 10 min, max speed >10.000 x g, 5 min Take 500 µL of supernatant and continue with step 3
3 BIND DNA		500 µL of supernatant (step 2) 250 µL Binding buffer Load the spin column (max. 600 µL) >10.000 x g, 1 min
4 WASH		500 µL of Wash buffer 1 >10.000 x g, 1 min 700 µL of Wash buffer 2 >10.000 x g, 1 min >10.000 x g, 3 min
5 ELUTE DNA		100 µL of Elution buffer (preheated at 70ºC) >10.000 x g, 1 min

TROUBLESHOOTING

Trouble	Possible Reason	Solution Suggest
Low DNA yield or low DNA purity	Inappropriate storage conditions	The Kit should be stored between +15 and +25^{o}C, except Proteinase K should be stored at -20^{o}C. The tube and bottle caps must be tightly sealed after each use to maintain the pH values and stability of the kit components, and to prevent contamination
	Chemicals and sample are not mixed well	The sample should be thoroughly mixed after each chemical addition
	Poor elution	Incubating the column with elution buffer for 2 minutes at 70 ºC may increase the yield Elute the DNA with 50 µL of elution buffer
No amplification after PCR/qPCR run or the enzymatic reactions are not working	Alcohol residue in DNA isolate	The remaining ethanol after washing steps should be removed by centrifuging the column at >10000 g for 3 minutes

QUALITY CONTROL

Each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

TRADEMARK, DISCLAIMER AND PRODUCT USE RESTRICTION

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. BPMR kit handbooks and user manuals can be requested from BPMR or your local distributor.

The use of this product signifies the agreement of any purchaser or user to the following terms: The kit must be used solely in accordance with the respective Instructions for Use. BPMR grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this kit except as described in the Instructions for Use. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

The kit components are intended exclusively for *in vitro* use, and for research purposes only! BPMR products are intended for general laboratory use only! Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results. It is strongly recommended to have dedicated areas, materials and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures. The workflow in the laboratory should proceed in a uni-directional manner, from the Extraction Area to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed the previous step. The user should always read all the instructions provided with the product before running the assay. Not mix reagents from different batches. Not use reagents from other manufacturer's products. Wear disposable gloves, laboratory coats when handling specimens and reagents. Use sterile pipette tips with filters. Waste must be treated and disposed of in compliance with the appropriate safety standards.

ADDITIONAL INFORMATION

For additional information, technical support or troubleshooting please contact: tech.support@biopremier.com

ORDERING INFORMATION

Biopremier offers a large selection of products. Visit www.biopremier.com or contact sales.support@biopremier.com for more detailed product information.