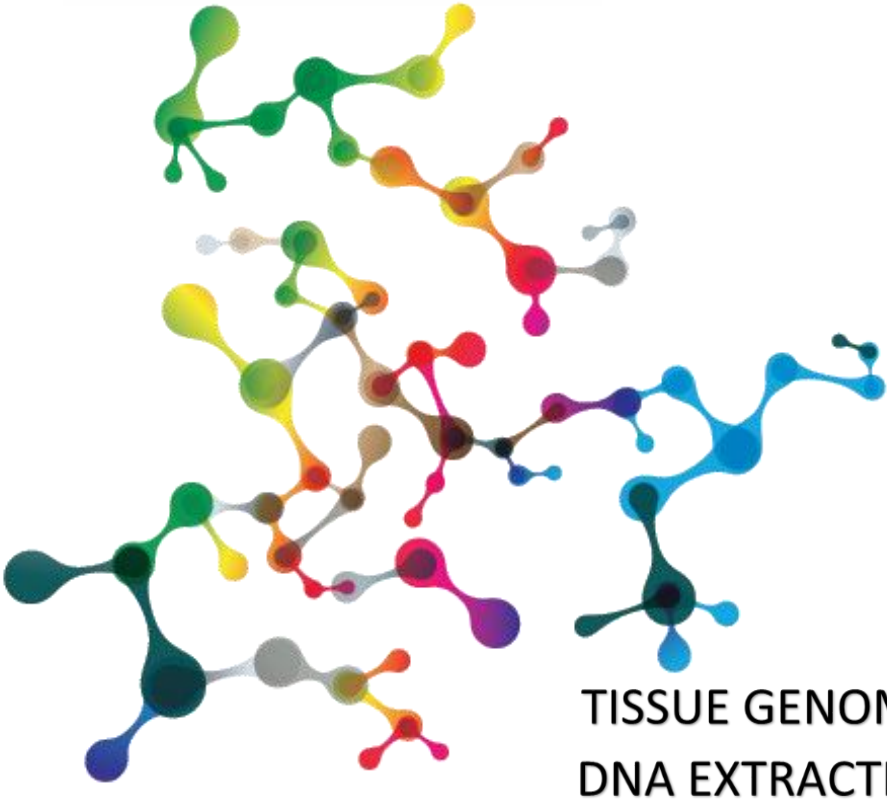
BIOPREMIER



TISSUE GENOMIC DNA EXTRACTION KIT

BIOPEXT-0605

CONTACT

BPMR – Production and Development, Lda.
Amadora INOVA
Rua Henrique de Paiva Couceiro, 10
2700 – 453 Falagueira – Venda Nova
Amadora, PORTUGAL
Tel: +351 211 451 410
E-mail: **sales.support@biopremier.com**
Website: **http://www.biopremier.com**

BPMR is certified ISO 9001:2015

INTRODUCTION AND PRODUCT DESCRIPTION

The Biopremier TISSUE Genomic DNA extraction Kit is designed for the rapid purification of highly pure genomic DNA from a wide variety of samples, including whole blood, culture cells, animal tissues, mouse tails, bacteria, yeats, clinic samples (serum, plasma, feces, orine), forensic samples and paraffin-embedded tissues.

Cell lysis is achieved by incubation of the sample in a solution containing SDS and proteinase K at 55ºC. Appropriate conditions for binding DNA to the glass fiber membrane are created by addition of chaotropic ions to the lysate. Contaminants are removed by efficient washing with wash buffer. Pure genomic DNA is finally eluted with an elution buffer and it's ready to use for subsequent reactions.

Applications:

- **For rapid purification of high-quality, ready-to-use DNA from various samples:**
  - **200 µL whole blood**
  - **200 µL clinic samples**
  - **200 µL buffy coat**
  - **10^4 – 10^6 culture cells**
  - **25-50 mg tissue**
  - **0.2-0.5 cm mouse tail**
  - **Paraffin-embedded tissues**

Features:

- **No organic extraction or alcohol precipitation.**
- **Complete removal of contaminants and inhibitors for reliable downstream applications.**
- **Typical yield: 25- 35 µg genomic DNA.**
- **Binding capacity: 60 µg.**
- **Elution volume: 50-200 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 unit		Storage
	50 preps	250 preps	
Tissue Lysis buffer	10 mL	50 mL	RT
Lysis binding buffer	10 mL	50 mL	RT
Proteinase K *	22 mg	105 mg	- 20ºC
Wash buffer 1 **	16.5 mL	82.5 mL	RT
Wash buffer 2 **	10 mL	50 mL	RT
Elution buffer	10 mL	50 mL	RT
Microspin columns	50 units	250 units	RT
Collection Tubes	100 units	500 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 %
Isopropanol

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 5 minutes at 37^{o}C and thoroughly shaken prior use.

PROCEDURE

PRELIMINARY PREPARATIONS

- ⚠ Make sure that Proteinase K and Wash Buffers were prepared according to "*Contents and Storage*" section, page 2
- ⚠ Preheat the Lysis binding buffer / Elution buffer at 70°C

1. GENOMIC DNA ISOLATION FROM WHOLE BLOOD, BUFFY COAT, CLINICAL SAMPLES and CULTURE CELLS

Homogenize sample

1. Pipette **200 µL** of your sample (whole blood, buffy coat or $10^4 – 10^6$ culture cells) to a microcentrifuge tube

If your sample volume is less than 200 µL add nuclease-free water to the final volume of the sample until you reach 200 µL.

Lyse cells step

2. Add **200 µL of Lysis binding buffer** (preheated) to the microcentrifuge tube, and add **20 µL of Proteinase K.** Vortex vigorously

3. Apply a short spin down and incubate for **10 minutes at 70°C.** Vortex one or twice time during incubation (optional)

4. Add **100 µL of Isopropanol.** Vortex vigorously

DNA binding step

5. Transfer **the lysate** into reservoir of a combined MicroSpin column-collection tube assembly

6. Centrifuge at **8.000 g for 1minute.** Remove the collection tube

Washing step

7. Place the MicroSpin column in a new collection tube and add **500 µl of Wash Buffer 1.**

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

9. Add **500 µl of Wash Buffer 2** and centrifuge at >**10.000 g for 1 minute**. Discard flow-through

10. Repeat the step 9: add **500 µl of Wash Buffer 2** and centrifuge at >**10.000g for 1 minute**. Discard flow-through

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

Elution step

12. Transfer the Microspin column to a new 1.5 ml microcentrifuge tube and pipette **200 µL of Elution Buffer** (preheated at 70^{o}C) onto the membrane. Incubateat room temperature for 2 minutes

    Decrease the volume of elution buffer if a higher DNA concentration is desired. For samples containing < 3 µg of DNA, eluting the DNA in 100 µl is recommended. For samples containing < 1 µg of DNA, eluting the DNA in 50 µl is recommended.

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

2. GENOMIC DNA ISOLATION FROM ANIMAL TISSUES

⚠ Before starting, preheat Lysis binding buffer and Elution buffer to 70°C

Sample preparation

1. Cut 25 mg of animal tissues in small pieces and place in 1.5 ml microcentrifuge tube.

Lyse cells step

2. Add **180 µL of Tissue Lysis buffer** to the microcentrifuge tube, and add **20 µL of Proteinase K.** Vortex vigorously

3. Apply a short spin down and incubate for **60 minutes (or overnight) at 55°C.** Vortex one- or twice-time during incubation (optional)

4. Add **200 µL of Lysis binding buffer** (preheated). Vortex vigorously

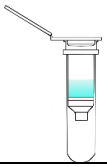
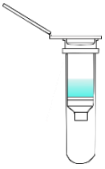
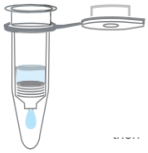
5. Apply a short spin down and incubate for **10 minutes at 70°C.** Vortex one or twice time during incubation (optional)

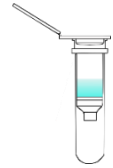
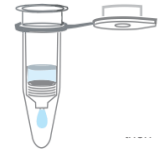
   If there are insoluble particles, centrifuge 5 minutes at maximum speed and pour the supernatant into a new tube.

6. Add **100 µL of Isopropanol.** Vortex vigorously

7. Proceed to DNA binding, Washing and Elution steps, in procedure #1 "GENOMIC DNA ISOLATION FROM WHOLE BLOOD, BUFFY COAT, CLINICAL SAMPLES and CULTURE CELLS" (pages 5 and 6)

WORKFLOW (1. BLOOD, BUFFY COAT, CLINICAL SAMPLES and CULTURE CELLS)

		PROCEDURE 1
1 SAMPLE PREPARATION		Pipette 200 µL of sample
2 LYSE CELLS		200 µL Lysis Binding Buffer + 20 µL Proteinase K; 70ºC, 10 min 100 µL Isopropanol
3 BIND DNA		Transfer the lysate to the Microspin column 8.000 x g, 1 min
4 WASH		500 µL of Wash buffer 1 >10.000 x g, 1 min 500 µL of Wash buffer 2 >10.000 x g, 1 min 500 µL of Wash buffer 2 >10.000 x g, 1 min >10.000 x g, 3 min
5 ELUTE DNA		50-200 µL of Elution buffer (preheated at 70ºC) >10.000 x g, 1 min

		PROCEDURE 2
1 SAMPLE PREPARATION		25 mg of animal tissue
2 LYSE CELLS		180 µL Tissue Lysis Buffer + 20 µL Proteinase K; 55ºC, 60 min – O/N 200 µL Lysis Binding Buffer; 70ºC, 10 min – O/N 100 µL Isopropanol
4 BIND DNA		Transfer the lysate to the Microspin column 8.000 x g, 1 min
5 WASH		500 µL of Wash buffer 1 >10.000 x g, 1 min 500 µL of Wash buffer 2 >10.000 x g, 1 min 500 µL of Wash buffer 2 >10.000 x g, 1 min >10.000 x g, 3 min
6 ELUTE DNA		50-200 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 +25ºC, except Proteinase K should be stored at -20ºC. 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

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