BIOPREMIER

BLOOD DNA EXTRACTION KIT

BIOPEXT-0606

CONTACT

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BPMR is certified ISO 9001:2015

INTRODUCTION AND PRODUCT DESCRIPTION

The Biopremier BLOOD DNA extraction Kit is designed for the rapid purification of pure genomic DNA from whole blood, serum, plasma, body fluids and dried blood spots.

The procedure involves the processing of either whole blood or DNA isolated from leukocytes, preceded by the lysis of erythrocytes using the RBC lysis buffer provided in the kit. Then, lysis is completed by incubation of whole blood in a solution containing chaotropic ions in presence of proteinase K at 70ºC. Appropriate conditions for binding DNA to the silica membrane are created by addition of ethanol to the lysate. Contaminants are removed by efficient washing with two different buffers. Pure genomic DNA is finally eluted under low ionic strength in a slightly alkaline elution buffer and it's ready to use for subsequent reactions.

Applications:

- **For rapid purification of high-quality, ready-to-use genomic, bacterial, viral DNA isolation from:**
  - **whole blood (human or animal fresh or frozen)**
  - **whole blood treated with citrate, EDTA and heparin**
  - **serum, plasma, buffy coat, platelets, body fluids, dried blood spots**

Features:

- **No organic extraction or alcohol precipitation.**
- **Complete removal of contaminants and inhibitors for reliable downstream applications.**
- **Typical yield: 4- 6 μg genomic DNA.**
- **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	
RBC Lysis buffer	50 mL	250 mL	RT
Tissue Lysis buffer	10 mL	50 mL	RT
Lysis binding buffer	15 mL	75 mL	RT
Proteinase K *	30 mg	2 x 75 mg	- 20ºC
Wash buffer 1 **	18 mL	90 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 %

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 Elution buffer at 70°C

SAMPLES

Whole blood samples: Refrigerated to 4°C immediately after collection and during transportation. They are stable for weeks at 4°C.

Serum samples: Blood should spin at 3000 rpm for 10 minutes. Blood serum samples are usually obtained using plain (non-anticoagulated) glass tubes to allow the blood to clot before harvesting the serum and transferring it to a smaller tube for transport. If the extraction in not possible within three days, the plasma and serum should be immediately frozen to -80°C or at least -20°C.

Plasma samples: Blood should spin at 3500 rpm for 15 minutes. The plasma samples should be promptly separated from cells and transferred to a 1.5 mL clean tube, while the intermediate layer containing white blood cells and platelets should not be transferred with the plasma. If the extraction in not possible within three days, the plasma and serum should be immediately frozen to -80°C or at least -20°C.

Buffy coat: Leukocyte-enriched fraction of whole blood. The preparation of a buffy-coat fraction from whole blood is simple and results in a yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Centrifuge whole blood at 2500 g for 10 minutes at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

1. GENOMIC DNA ISOLATION FROM WHOLE BLOOD (leukocytes)

This protocol is for the purification of DNA from leukocytes by performing a selective lysis of the erythrocytes with the RBC Lysis Buffer.
If only genomic or mitochondrial DNA is required, this is the ideal method because it produces better results in terms of quality and performance.

⚠ Before starting, preheat Elution buffer to 70°C

Sample preparation

1. Pipette **300 µL** of blood into a 1.5 mL microcentrifuge tube

Lyse cells step

2. Add **900 µL of RBC lysis Buffer.** Vortex vigorously and incubate at room temperature for 10 minutes

3. Centrifuge at **maximum speed for 1minute.** Eliminate the supernatant by decanting and leave 10-20 µl of residual liquid. Vortex the microtube to resuspend the pellet

   It is recommended to decant the supernatant rather than using a micropipette, as the latter may inadvertently aspirate the small, non-visible cell pellet.

4. Add **180 µL of Lysis Tissue Buffer** to the microcentrifuge tube, and add **25 µL of Proteinase K.** Vortex vigorously

5. Apply a short spin down and incubate for **15 minutes at 56°C**

6. Add **200 µL of Lysis/ Binding Buffer.** Vortex vigorously and incubate for **10 minutes at 70°C**

7. Add **200 µL of Ethanol (96-100%).** Vortex vigorously

DNA binding step

8. Transfer **500 µL of the sample** to a combined Spin column-collection tube assembly

9. Centrifuge at **8.000 g for 1minute.** Discard flow-through

10. Repeat the steps 8 and 9 for the remaining sample

11. Remove and discard the collection tube

Washing step

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

13. Centrifuge at **8.000 g for 1 minute.** Discard flow-through

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

15. Repeat the step 14: add **500 µl of Wash Buffer 2** to the reservoir and centrifuge at >**10.000g for 1 minute**. Discard flow-through

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

Elution step

17. Transfer the Spin column to a new 1.5 ml microcentrifuge tube and pipette **50-200 µL of Elution Buffer** (preheated at 70ºC) onto the membrane. Incubateat room temperature for 2 minutes

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

18. 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 WHOLE BLOOD, BUFFY COAT AND BIOLOGICS FLUIDS

⚠ Before starting, preheat Elution buffer to 70°C

⚠ This protocol is for purification of total (genomic, mitochondrial and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes and body fluids

Sample preparation

1. Pipette **300 µL** of sample into a 1.5 mL microcentrifuge tube

   Use up to 300 µL whole blood, plasma, serum, buffy coat or body fluids. For volumes less than 300 µL, add PBS to adjust the volume.
   If purifying DNA viruses, we recommend starting with 300 µL serum or plasma.

Lyse cells step

2. Add **300 µL of Lysis / Binding Buffer** to the microcentrifuge tube, and add **25 µL of Proteinase K.** Vortex vigorously (10 – 20 sec.). Incubate for **15 minutes at 70°C**

   The lysate should become brownish during incubation. For older or clotted blood samples, increase incubation time with Proteinase K (up to 30 minutes) and vortex once or twice vigorously during incubation.

3. Add **300 µL of Ethanol (96-100%).** Vortex vigorously

DNA binding step

4. Transfer **500 µL of the sample** to a combined Spin column-collection tube assembly

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

6. Repeat the steps 4 and 5 for the remaining sample

7. Remove and discard the collection tube

8. Proceed to Washing and Elution steps, in procedure #1 “GENOMIC DNA ISOLATION FROM WHOLE BLOOD (leukocytes)” (page 7)

3. GENOMIC DNA ISOLATION FROM DRIED BLOOD SPOTS

⚠ Before starting, preheat Elution buffer to 70°C

Sample preparation

1. Place 3 punched-out circle from dried blood spot into 1.5 mL microcentrifuge tube

2. Add **200 µL** of PBS and vortex vigorously. Incubate for **10 minutes at 85°C.** Briefly centrifuge to remove drops from inside the lid

Lyse cells step

3. Add **200 µL of Lysis / Binding Buffer** to the microcentrifuge tube, and add **25 µL of Proteinase K.** Vortex vigorously (10 – 20 sec.). Incubate for **60 minutes at 70°C**

4. Add **200 µL of Ethanol (96-100%).** Vortex vigorously

DNA binding step

5. Transfer **500 µL of the sample** to a combined Spin column-collection tube assembly

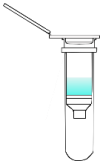
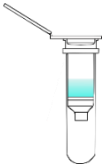
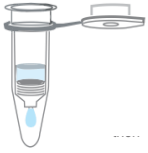
6. Centrifuge at **10.000 g for 1minute.** Discard flow-through

7. Repeat the steps 4 and 5 for the remaining sample

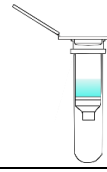
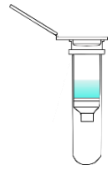
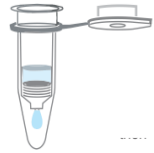
8. Remove and discard the collection tube

9. Proceed to Washing and Elution steps, in procedure #1 “GENOMIC DNA ISOLATION FROM WHOLE BLOOD (leukocytes)” (page 7)

WORKFLOW (1. GENOMIC DNA ISOLATION FROM WHOLE BLOOD (leukocytes)

		PROCEDURE 1
1 SAMPLE PREPARATION		Pipette 300 µL of sample
2 LYSE CELLS		900 µL RBC Lysis Buffer RT, 10 min > 10.000 x g, 1 min 180 µL Lysis Tissue Buffer + 25 µL Proteinase K; 56ºC, 15 min 200 µL Lysis / Binding Buffer 70ºC, 10 min 200 µL Ethanol (96-100%)
3 BIND DNA		Transfer the lysate to the Spin column 8.000 x g, 1 min
4 WASH		500 µL of Wash buffer 1 8.000 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

WORKFLOW (2. Whole blood, Buffy coat and Biologics fluids)

	PROCEDURE 2
1 SAMPLE PREPARATION	Pipette 300 µL of sample
2 LYSE CELLS 	300 µL Lysis Tissue Buffer + 25 µL Proteinase K; 70ºC, 15 min 200 µL Ethanol (96-100%)
4 BIND DNA 	Transfer the lysate to the Spin column 8.000 x g, 1 min
5 WASH 	500 µL of Wash buffer 1 8.000 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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