BehPrep Genomic DNA Extraction Kit

Cat. No: BPGD100 100 reactions
Store kit at room temperature
Expiration: 1 year

Small-Scale Preparations of DNA Isolation

Content:

	100 Preps
Spin Column	100
Collection Tube	300
Proteinase K	2 ml
LB Buffer	22 ml
WB1 Buffer	22 ml
WB2 Buffer	16 ml
EB Buffer	15 ml

Storage and stability

Spin columns of the kit are packed in closed bags and show full performance in this state at room temperature (18-25°C) until expiration date. All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved. The buffers can show a slight yellow color. This will have no impact on the function of the buffer.
Spin columns are packed in closed bags, please take care that columns should be used after once opened. Please take care that columns, once opened, should be used instantly. Close bottles immediately after use.

Kit Description

The Behprep Genomic DNA Extraction Kit was designed to purify genomic DNA from blood and buffy coat. Purification is based on spin column silica using Behprep's proprietary Silica membrane

as the separation matrix. Behprep's slica binds DNA under high salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with PCR and Southern Blot analysis.

Important notes: please read before using

- ✓ Wear gloves in all steps of procure.
- ✓ Equilibrate samples to room temperature.
- ✓ Equilibrate all Buffer to room temperature before use.
- ✓ Homogenize all buffer before use.
- ✓ All centrifugation steps are carried out at room temperature (15–25°C).
- ✓ Always vortex the Proteinase K before use.
- ✓ For best results, the use of whole blood collected into tubes containing an anticoagulant is highly recommended.
- ✓ Both fresh and frozen anticoagulated blood may be used with this procedure. Ensure that frozen blood is thawed at room temperature prior to starting the protocol.
- ✓ For blood containing Gram positive bacterial pathogens, prepare a 400 mg/mL stock solution (approximately 1.7 x 107 units/mL) of lysozyme as per supplier's instructions.

Reagents and equipment required (but not supplied)

- Pipets and pipet tips (RNA and DNA free)
- 1.5 ml Microtube (RNA and DNA free)
- Vortex
- Micro pipettors
- Absolute Ethanol
- Microcentrifuge
- NaCl 0.9% solution or PBS (For samples <200 µl)

Warnings and precautions

- ➢ Wear gloves in all steps of procure.
- ➢ The Buffer LB contains guanidine salt, and should be handled with care. Guanidine salt forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.
- ➢ Buffers are toxic and irritant, they should be open in fume hood.
- ➢ Avoid contact any kit reagents with skin & eyes.
- ➢ Follow biohazard safety procedure when handling human samples.

Samples

This kit is for use for extracting DNA from whole blood.

Buffer preparation (working solution)

the appropriate amount of ethanol (96–100%) as indicated on the bottle and in Table 1. WB1 Buffer is stable for 1 year when stored closed at room temperature (15–25°C), but only until the kit expiration date.

- Preparation of WB1 Buffer

No. preps	WB1 concentrate	Ethanol	Final volume
100	22 ml	29 ml	51 ml

Table 1

❖ WB2 Buffer

WB2 Buffer is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) to WB2 Buffer concentrate as indicated on the bottle and in Table 2.

WB2 Buffer is stable for 1 year when stored closed at room temperature (15–25°C), but only until the kit expiration date.

- Preparation of WB2 Buffer

No. preps	WB2 concentrate	Ethanol	Final volume
100	16ml	37ml	53 ml

Table 2

Protocol

1. Pipet 20 µl **Proteinase K** into a 1.5 ml microcentrifuge tube. Add 200 µl **sample**. If the sample volume is less than 200 µl, add the appropriate volume of PBS. Add 200 µl **LB Buffer**. Mix thoroughly by vortexing, Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the lid and Incubate at 56°C for 12 min. Then add 250 µl **ethanol (96–100%).** Mix thoroughly by vortexing. Briefly centrifuge the tube to remove drops from the lid.

2. Pipet the mixture onto the spin column (in a 2 ml collection tube) and centrifuge at 8000 rpm for 1 min. discard the flow-through and collection tube.

   Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

3. Add 500 µl **WB1 Buffer** and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through and place back the spin column in the collection tube.

   Note: This step increases kit performance when processing inhibitory samples.

4. Add 500 µl **WB2 Buffer** and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through and place back the spin column in the collection tube.

   **WB carryover into the elute may cause problems in downstream applications.*

5. Place back the spin column in the collection tube. Centrifuge at full speed (20000 x g, 14000 rpm) for 3 min to dry the membrane completely. Then discard the collection tube and flow-through.

6. Place the spin column in a clean 1.5 ml clean microcentrifuge tube (not provided). Apply 20–150 µl **EB Buffer** to the center of the membrane. Close the lid and incubate at room temperature for 5 min. Centrifuge at full speed, 20000 x g (14000 rpm) for 1 min.

Specifications

- **High Yield**: up to 10 µg of Genomic DNA
- **High Quality DNA**: A260/A280 = 1.8-1.9
- **Sample:** 100 to 300 µl of whole blood and buffy coat (5 x 10_6 WBC)
- **Operation time:** within 25 minutes (manual)
- **Storage:** dry at room temperature (15-25ºC), Proteinase K should be stored dry at -20ºC for extended periods.

Quality control

All the component and lot numbers (Lot NO.) of each kit in the quality control unit of Vista Biotechnology Behgene have been evaluated according to predetermined criteria to ensure the accuracy of the product.

Address:

Postal code:

Phone:

Web site: