For life science research only.
Not for use in diagnostic procedures.

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BehMag Blood Extraction Kit

Small-Scale Preparations of DNA Isolation based on Magnetic beads

Components

Catalog No.	25 preps	50 preps
LB Buffer	5ml	10ml
Magnetic Beads	1.5.ml	3 ml
Proteinase K	0.8 mL	1.2 mL
WB Buffer	15ml	30ml
EB Buffer	5ml	10ml

Stored at room temperature

Expiration: 1 year

Companion Device----------

Magnetic separation rack

Equipment & Reagents to be supplied by user----------

- Pipets and pipet tips
- 1.5 ml Microtube
- Vortex
- Dry Heat Block/ Water Bath
- Micropipettors
- 70% ethanol (prepare fresh)
- 96-100% Isopropyl
- Nuclease-free water

*The Buffer TN contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Precautions and Disclaimers----------

- This kit has been designed for research purposes only. It is not intended for human or diagnostic use.
- Avoid freeze/thaw cycles and centrifugation which could damage the beads.
- Be sure to mix well before using magnetic beads, can be vortexed about 10 seconds.
- Vortex samples 10 seconds before adding

Description ----------

The BehMag blood DNA Extraction Kit was designed to purify genomic DNA from blood and buffy coat. Magnetic beads with uniform particle size efficiently bind DNA, resulting in high yields of DNA with minimal RNA, proteins, and other cellular contaminants. The kit is

intended for manual purifications using a magnetic separator. This kit can be easily adapted to automated magnetic bead separation instruments and workstations. The uniform size and surface area of BehMag magnetic beads ensure highly reproducible results and improved performance compared to magnetic beads from alternative suppliers. Bead mean diameter is ~0.1 μm, Bead concentration is 20 mg/mL and Binding capacity varies with sample type. Blood and buffy coat. This product is for research use only and is not intended for therapeutic or diagnostic applications.

Specifications----------

- **High Yield**: up to 10 μg of Genomic DNA
- **High Quality DNA**: A260/A280 = 1.8-2.0
- **Sample:** 100 to 400 μl of whole blood and buffy coat (5 x 10^6 WBC)
- Manual or automated DNA isolation
- **Operation time:** within 25 minutes (manual)
- **Storage:** dry at room temperature (15-25ºC), Protease should be stored dry at 2-8ºC for extended periods.

Procedure ----------

Notes prior to use:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.

- Always vortex the Magnetic Bead Suspension before use.

- Always vortex the Proteinase K before use.

- Preheat the incubator(s) according to the protocol (37ºC, 55°C or 65°C).

- Prepare a working concentration of the Solution WN by adding 24 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated Solution WN. This will give a final volume of 42 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

- 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

1. Sample Preparation

NOTE: For DNA isolation from blood containing Gram positive bacterial pathogens, please see Appendix A for Sample Preparation.

a. Add 20 μL of Proteinase K (vortex before use) to a microtube.

b. Transfer 20 - 200 μL of blood sample to the tube containing Proteinase K.

c. Add 300 μL of LB Buffer to the blood and mix well by vortexing for 10 seconds.

d. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

e. Incubate at 55ºC for 10 minutes. Note: If any debris is present in the sample, centrifuge for 2 minutes at 14,000 x g (~14,000 RPM) to precipitate. Transfer the clean supernatant to a microtube prior to Step f.

f. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

g. Add an equal volume of 96-100% Ethanol (100 μL of ethanol is added to every 100 μL of lysate) to the sample and mix well by vortexing for 10 seconds.

h. Add 40 μL of Magnetic Bead Suspension (vortex prior to use) to the lysate collected above.

i. Incubate at room temperature for 10 minutes. Occasionally invert the tube.

j. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

2. Blood DNA Isolation

a. Assemble a magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 30 sec.

b. Aspirate and discard supernatant without touching the magnetic beads.

c. Remove the sample tube from the magnetic rack and gently add 300 µL of Solution WB. Resuspend by vortexing or pipetting for 30 sec.

d. Place the sample tube on the magnetic rack and allow to sit for 30 sec.

e. Aspirate and discard supernatant without touching the magnetic beads.

f. Remove the sample tube from the magnetic rack and gently add 500 µL of freshly prepared 70% ethanol. Resuspend by vortexing or pipetting and incubate at room temperature for 30 sec.

g. Place the sample tube on the magnetic rack and allow to sit for 30 sec.

h. Aspirate and discard supernatant without touching the magnetic beads.

i. Repeat Steps 2f – 2h for a second wash step. 5 Note: Remove as much of the 70% ethanol in the sample tube as possible by pipetting.

j. Incubate the open tube at 60˚C for 5 minutes to dry the magnetic beads.

k. Remove the sample tube from the magnetic rack and add 50-100µL of EB Buffer. Mix by vortexing and incubate at 60˚C for 15 minutes.

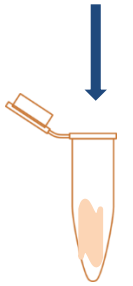
l. Briefly vortex and place sample tube on the magnetic rack and allow to sit for 30 sec.

m. Carefully transfer the elution to a fresh 1.5 mL elution tube without touching the magnetic beads.

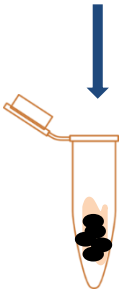
The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long-term storage

Flow Chart

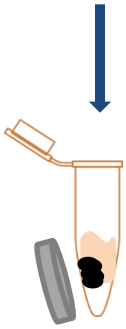
Obtain anticoagulated blood sample and transfer into a tube containing Proteinase K.



Adding LB buffer and incubating



Adding Magnetic beads

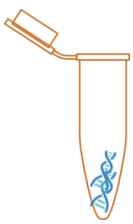


Applying Magnetic field
Discarding the supernatant

Washing the beads

Adding EB buffer

Incubate at 55°C for 10 min



Transferring supernatant to a clean tube.