MANUAL

RayBio® Blood Genome Extraction Kit (Magnetic Bead Method)

ISO 13485:2016

Catalog #: NAE-BGEN-MAG

Purpose

This kit is used for the extraction, enrichment, and purification of genomic DNA from human blood samples.

Kit Components (100 sample kit)

Store kit in a dry environment at 2 – 30°C for up to 24 months. Kit must be used within the validity period to ensure correct performance.

Component	Catalogue #	Size	Quantity
Lysis Buffer B	NAE-LB-B	90 mL/bottle	1 bottle
Proteinase K	NAE-PK	1 mL/vial	2 vials
Magnetic Bead Solution A	NAE-MAG-A	1 mL/vial	1 vial
Wash Buffer A	NAE-WB-A	60 mL/bottle	1 bottle
Wash Buffer B	NAE-WB-B	60 mL/bottle	1 bottle
Wash Buffer C	NAE-WB-C	60 mL/bottle	1 bottle
Elution Buffer A	NAE-EB-A	1.5 mL/vial	4 vials

Note: Do not mix reagents from different lots.

Required Materials (NOT INCLUDED)

1. Magnetic frame
2. Tabletop centrifuge
3. Vortex Mixer
4. Water bath or dry heat block
5. Pipettes and sterile nuclease free pipette tips (barrier tips recommended)
6. Nuclease free microfuge and conical tubes
7. 100% isopropanol

General Considerations

1. Please read this manual carefully before the experiment.
2. All samples should be regarded as potentially infectious and handled with extreme caution. Measures should be taken to minimize the risk of laboratory transmission based on risk assessment when testing any samples. These precautions, at a minimum, should include proficiency and competency testing, appropriate PPE, hazardous waste disposal, avoiding aerosols, and using effective disinfectants (quaternary ammonium compounds and 0.5% bleach). It is also recommended that extractions be performed in an appropriate biosafety cabinet.
3. Please use calibrated pipettes and nuclease free reaction tubes centrifuge tubes, conical tubes, and pipette tips.
4. Laboratory management should strictly follow the management standards for PCR gene amplification.

Sample Requirements

1. Sample type: Human anticoagulant whole blood.
2. Sample volume: 0.1 mL
3. Sample storage: After collection, blood samples can be stored for 7 days at 2°C to 8°C. If not planning to use in this time frame, store at or below -20oC immediately after collection for no longer than 1 year.

Protocol

1. **Reagent preparation**: Ensure that Lysis Buffer B is completely thawed and free from crystallization.

   Note: *If crystals are apparent, warm Lysis Buffer B to 30°C and gently vortex until crystals dissipate.*
2. **Sample preparation**: Thaw blood sample at room temperature (15 to 30°C) for 30 minutes immediately prior to use. Once thawed, place blood samples on ice.

   Note: *Proceed to step 3 within 60 minutes of thawing samples.*
3. **Nucleic acid extraction**

   3.1. Lysis:

   3.1.1. In a prelabeled 1.5 mL centrifuge tube, add 100 µL blood sample, 20 µL Proteinase K, and 150 µL Lysis Buffer B in order. Tightly seal the tube, vortex to mix well, spin briefly at low speed, and

incubate at 56°C for 10 minutes.

3.1.2. Add 200 µL of 100% isopropanol and 10 µL Magnetic Bead Solution A to the above tube. Vortex to mix well and centrifuge briefly.

3.1.3. Incubate at room temperature for 10 minutes. Vortex for 10 seconds every 2 minutes.

3.2. Nucleic acid washing:

3.2.1. Centrifuge briefly at low speed and place on magnetic stand for 2 minutes. Discard the clarified liquid waste.

3.2.2. Add 600 µL Wash Buffer A, vortex for 10 seconds, and centrifuge briefly. Place tube on magnetic stand for 2 minutes. Discard the clarified liquid waste.

3.2.3. Add 600 µL Wash Buffer B, vortex for 10 seconds, and centrifuge briefly. Place tube on magnetic stand for 2 minutes. Discard the clarified liquid waste.

3.2.4. Add 600 µL Wash Buffer C, vortex for 10 seconds, and centrifuge briefly. Place tube on magnetic stand for 2 minutes. Discard the clarified liquid waste.

3.2.5. Open tube in clean environment and dry at room temperature for 2 minutes.

***Note**: We recommend doing this step in a clean, sterilized PCR hood.*

3.3. Nucleic acid elution:

3.3.1. Add 50 – 100 µL Elution Buffer A to the 1.5 mL centrifuge tube from Step 3.2. Tightly seal tube and then vortex thoroughly.

3.3.2. Incubate sample at 56°C for 3 minutes. Vortex for 5 seconds every minute.

3.3.3. Place tube in magnetic rack for 2 minutes. Transfer clarified liquid to a new 1.5 mL centrifuge tube.

***Note:** The clarified liquid contains the extracted nucleic acid. Do not discard!*

3.3.4. Extracted nucleic acid can be used immediately or stored below -20°C for future use.

Product Performance

Repeatability: Precision testing showed that the coefficient of variation (CV) of the precision Cq values within lots is less than 5%.

Limitations

1. Assay performance has been validated with blood samples collected in K2 EDTA. No testing was completed for other blood sample collection modalities.

References

1. Vogelstein B et al. Preparative and analytical purification of DNA from agarose. Proc Natl Acad Sci, 1979, 76(2), 615-619.
2. Maurice Stroun *et al*. Isolation and characterization of DNA from the plasma of cancer patients. European Journal of Cancer and Clinical Oncology, 1987, 23(6), 707-712.
3. M. Fleischhacker, B. Schmidt. Circulating nucleic acids (CNAs) and cancer—A survey. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 2007, 1775(1), 181-232.