MANUAL

RayBio® Peripheral blood cell-free nucleic acid extraction kit (Magnetic Bead Method)

ISO 13485:2016

Catalog #: NAE-PBNA-MAG

Purpose

This kit is used for the extraction, enrichment, and purification of peripheral blood cell-free nucleic acids from human plasma samples.

Kit Components (50 sample kit)

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

Component	Catalogue #	Size	Quantity
Lysis Buffer A	NAE-LB-A	90 mL/bottle	1 bottle
Protease K	NAE-PK	10 mL/bottle	1 bottle
Magnetic Bead Solution A	NAE-MAG-A	1 mL/vial	1 vial
Binding Buffer A	NAE-BB-A	80 mL/bottle	2 bottles
Wash Buffer A	NAE-WB-A	50 mL/bottle	1 bottle
Wash Buffer B	NAE-WB-B	30 mL/bottle	1 bottle
Wash Buffer C	NAE-WB-C	30 mL/bottle	1 bottle
Elution Buffer A	NAE-EB-A	2 mL/vial	2 vials

Note: Do not mix reagents from different lots.

Required Materials (NOT INCLUDED)

1. Magnetic frame
2. Centrifuge (>12,000 x g)
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% ethanol

General Considerations

1. Please read this manual carefully before the experiment.
2. It is recommended that genomic DNA be used immediately after purification. If this is not possible, store purified genomic DNA at -20°C.
3. 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.
4. Please use calibrated pipettes and nuclease free reaction tubes centrifuge tubes, conical tubes, and pipette tips.
5. Laboratory management should strictly follow the standards for PCR gene amplification.

Sample Requirements

1. Sample type: Human plasma.
2. Sample collection: Use K2 EDTA or nucleic acid free blood tubes to collect 10 mL of blood according to the manufacturer's recommendations. Following collection, K2 EDTA blood collection tubes should be stored at 2-8°C and treated within 24 hours after collection (do not freeze). Nucleic acid free blood collection tubes can be stored at 15-25°C for up to 72 hours before analysis.
3. Sample preparation:

   3.1 Spin blood collection tube at 1350 ± 150 RCF for 12 minutes.

   Note: Ensure that the brake function is turned off to prevent damage to the hemostatic cell layer.

   3.2 Aseptically transfer plasma to an appropriately sized centrifuge tube and spin again at 1350 ± 150 RCF for 12 minutes.

   3.3 Aseptically transfer 3.5 mL of plasma into a new labeled centrifuge tube.

   3.4 Plasma samples can be used immediately or stored at 2 to 8°C for 24 hours, -20°C for one month, or -70°C for six months.

Protocol

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

   Note: *If crystals are apparent, warm Lysis Buffer A to 30°C and gently vortex until crystals dissipate.*

2. **Sample preparation**: Thaw plasma sample at room temperature (15 to 30°C) for 30 minutes immediately prior to use. Once thawed, place plasma samples on ice.

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

3. **Nucleic acid extraction**

   3.1. Lysis: In a prelabeled centrifuge tube, mix 100 µL Protease K, 900 µL Lysis Buffer A, and 1 mL of plasma sample. Tightly seal tubes, vortex to mix well, and heat to 56°C for 10 minutes in a preheated water bath or dry heat block.

   Note: *This kit can be used to extract nucleic acid from plasma sample volumes from 0.2 mL to 2 mL. Please scale up/down amount of Protease K and Lysis Buffer A depending on the plasma sample volume.*

   3.2. Nucleic acid binding:

   3.2.1. Transfer lysate (2 mL total volume) to a 5 – 15 mL tube.

   Note: *Lysate volume is dependent on starting plasma sample volume.*

   3.2.2. Add 1.6 mL Binding Buffer A and then 1.3 mL 100% ethanol (in order) to the lysate. Tightly seal the tubes and then vortex well to mix.

   Note: *Above volumes are for 1 mL plasma sample volume. Please scale up/down depending on the starting plasma sample volume.*

   3.2.3. Add 10 µL Magnetic Bead Solution A. Tightly seal tubes and then vortex well to mix.

   Note*: Above volume is for 1 mL plasma sample volume. Please scale up/down depending on the starting plasma sample volume.*

   3.2.4. Incubate at room temperature for 10 minutes. Vortex for 5 seconds every 2 minutes.

   3.2.5. Place tube onto a magnetic frame for 3 minutes and discard the clarified liquid waste.

   3.3. Nucleic acid washing:

   3.3.1. Add 1 mL Wash Buffer A to the tube from Step 3.2, vortex well, and transfer to new 1.5 mL

centrifuge tube. Place on magnetic stand for 2 minutes and discard the clarified liquid waste.

3.3.2. Add 0.6 mL Wash Buffer B, vortex well, briefly centrifuge at low speed. Place on magnetic stand for 2 minutes and discard the clarified liquid waste.

3.3.3. Add 0.6 mL Wash Buffer C, vortex well, briefly centrifuge at low speed. Place on magnetic stand for 2 minutes and discard the clarified liquid waste.

3.3.4. 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.*

3.4. Nucleic acid elution:

3.4.1. Add 50 – 100 µL Elution Buffer A to the 1.5 mL centrifuge tube from Step 3.3. Tightly seal tube and then vortex thoroughly.

3.4.2. Incubate sample at 56°C for 3 minutes in water bath or dry heat block. Vortex for 5 seconds every minute.

3.4.3. Place tube in magnetic stand 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!*

3.4.4. Extracted nucleic acid can be used immediately or stored below -20°C for up to 24 hours.

Product Performance

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

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.