MANUAL

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ISO 13485:2016

RayBio® Cell Genome Extraction Kit (Magnetic Bead Method)

Catalog #: NAE-CGEN-MAG

Purpose

This kit is used for the extraction, enrichment, and purification of human genomic DNA from human swab 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 B	NAE-LB-B	10 mL/bottle	1 bottle
Proteinase K	NAE-PK	1 mL/vial	1 vial
Magnetic Bead Solution A	NAE-MAG-A	0.5 mL/vial	1 vial
Binding Buffer A	NAE-BB-A	16 mL/bottle	1 bottle
Wash Buffer A	NAE-WB-A	30 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	1.5 mL/vial	2 vials

Note: Do not mix reagents from different lots.

Required Materials (NOT INCLUDED)

1. Magnetic frame
2. Centrifuges (>12,000 x g; low speed tabletop)
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
8. Ultrapure distilled water

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 standards for PCR gene amplification.

Sample Requirements

1. Sample type: Human swab in cell preservation solution.
2. Sample volume: 3 mL
3. Sample storage: Samples can be stored at 2ºC to 8ºC for 7 days. If not planning to use samples in this time frame, samples should be stored at or below -20ºC immediately after collection. Samples stored in this way are stable for up to 1 year from collection. Avoid freeze/thaw cycles.

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 30ºC and gently vortex until crystals dissipate.*
2. **Sample preparation**: Store the swab in cell preservation solution according to manufacturer's instructions.

   2.1. Transfer 3 mL of the cell preservation solution to a new, labeled 1.5 mL microcentrifuge tube.

   2.2. Centrifuge at 14000 RCF for 2 minutes and discard supernatant by aspiration or decanting.

   2.3. Resuspend pellet with 200 µL ultrapure distilled water.
3. **Nucleic acid extraction**

   3.1. Lysis:

3.1.1. Mix 20 µL Proteinase K and 180 µL Lysis Buffer B (in order) to the sample (from step 2). Vortex to mix well. Incubate at 56°C for 10 minutes.

3.2. Nucleic acid binding:

3.2.1. Add 320 µL Binding Buffer A and 260 µL 100% Ethanol to the lysed cell sample. Centrifuge briefly at low speed.

3.2.2. Add 10 µL Magnetic Bead Solution A and vortex for 10 seconds.

3.2.3. Incubate at room temperature for 10 minutes. Inverting 5 times every 2 minutes.

3.2.4. Centrifuge briefly and place on magnetic stand for 2 minutes. Discard the clarified liquid waste.

3.3. Nucleic acid washing:

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

3.3.2. 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.

3.3.3. 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.

3.3.4. 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.

3.4.2. Tightly seal the tube and then vortex thoroughly.

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

3.4.4. 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!*

3.4.5. Extracted nucleic acid can be used immediately or stored below -20°C for up to one year.

Limitations

Assay performance has been validated with a swab stored in cell preservation solution. No other sample collection methods have been tested.

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.