Nucleic Acid Extraction Kit

Product Code : PT/NCV/001

Intended Use:

For isolation of nucleic acid from various samples like fresh and frozen plasma, serum, nasopharyngeal and oropharyngeal swabs, sputum samples collected in Viral Transport Medium or Inactivation Transport Medium.

Introduction

The kit is designed for efficient isolation of Nucleic Acid from multiple viral samples in less than 20 minutes. A very convenient alternative of conventional methods of Nucleic Acid =isolation like CsCl ultracentrifugation and trizol based extraction which are laborious and time consuming.

The nucleic acid extraction kit has been optimized to stabilize the viral RNA/DNA and ensures proper nucleic acid isolation for molecular analysis. The kit can be used for various applications like- real time PCR, cDNA synthesis, northern blotting, primer extension, poly A + RNA selection and microarrays.

Principle

Promea Nucleic Acid Extraction Kit is based on the principle of selective binding of RNA/DNA in silica-based membrane and washing out of unwanted material by centrifugation. A proprietary designed high salt buffer facilitates binding of RNA/DNA with silica membrane and elution of the same using low salt buffer condition. The viral membrane is first lysed using a highly denaturing chaotropic reagent. The free RNA/DNA then binds to column with the help of ethanol. Ethanol provides appropriate buffer conditions. The unwanted contaminants are then washed of using proprietary washing buffers. High quality nucleic acid is subsequently extracted for downstream applications.

Kit Contents:

Reagents provided	100 preps
Lysis Buffer	35 ml
Concentrated Wash Buffer	16 ml x 2
Elution Buffer	12 ml
Carrier RNA	1400 µg
Spin Columns placed inside uncapped collection tubes	100 nos
Collection Tubes	100 nos x 2
Kit Insert	1 no

Materials required but not provided:

- Microcentrifuge tubes
- Microcentrifuge
- Pipettes
- Filter Tips to avoid aerosol contamination
- Absolute Ethanol

Precautions:

- Use Nuclease free plastic ware to avoid degradation.
- Follow precautionary Biosafety procedures while handling Clinical specimens.
- If any precipitate is seen in PLB, keep the solution at 60℃ for 10 min to ensure dissolution of the precipitate.
- Always wear gloves while handling reagents and RNA samples to prevent RNase contamination from dusty laboratory equipment or the surface of skin.
- Use sterile, disposable plasticware and autoclavable pipettes to avoid contamination.
- Electrophoresis tanks should be cleaned with detergent solution then with RNase free water and then rinse with ethanol and allowed to dry.
- Solution should be treated with 0.1% DEPC.

Storage condition:

- NAEK can be stored at room temp (25-30℃) for up to 1 year.
- Recommend storage of the reconstituted carrier RNA at -20℃ in aliquots.

Procedure:

- Add 330 µL lysis and 5 µL carrier RNA to 140 µL of sample in a collection tube and vortex well.
- Add 330 µL of absolute ethanol and transfer lysate to a spin column.
- Centrifuge the spin column at 8000 rpm for 1 minute.
- Discard the flow through and add 500 µL of wash buffer.
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- Discard the flow through and add 500 µL of wash buffer.
- Centrifuge at 8000 rpm for 1 minute.
- Discard flowthrough and dry spin at 10000 rpm for 2 minutes.
- Transfer spin column to 1.5 ml Eppendorf tube and add 50 µL of elution buffer.
- Incubate for 1 minute.
- Centrifuge at 8000 rpm for 1 minute
- Transfer the isolated nucleic acid for proper storage.
- Discard the used tubes and spin column after decontamination.

General Instruction:

- Add 48 ml of absolute ethanol to each of the concentrated wash buffer before use.
- Add 500 µL of Elution Buffer to carrier RNA Vial.
- Use the second concentrated wash buffer only after consumption of the first bottle.
- Ensure ethanol is added to PWB concentrate before use.

Reconstitution of carrier RNA:

- Add 500 µL of Elution Buffer to carrier RNA Vial.
- Dissolve carrier RNA thoroughly by pipetting.
- Storage of reconstituted carrier RNA at -20℃ in aliquots is recommended to avoid repeated freeze and thaw.

Disposal:

User must ensure safe disposal by autoclaving and /or incineration of used or unusable preparations of this product. Follow established laboratory procedures in the disposal of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with correct laboratory techniques.

References:

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Verheyen J, Kaiser R, Bozic M, et al. Extraction of viral nucleic acids: comparison of five automated nucleic acid extraction platforms. J Clin Virol 2012; 54:255e9.

Chandler DP, Griesemer SB, Cooney CG, et al. Rapid, simple influenza RNA extraction from nasopharyngeal samples. J Virol Methods 2012; 183:8e13

ISO 13485:2016 cGMP

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Symbols

REF	Catalogue No.	LOT	Batch No.		Consult instruction for use
	Manufacturer		Expiry date		Keep away from direct sunlight
	Manufacturing date		Keep dry		Do not use if box open or damaged
	Storage temperature		Sufficient for		
IVD	In Vitro Diagnostics		Caution		