

**MBIN013****Insta NX® Viral RNA Purification Kit****Kit Contents**

Product Code	Reagents provided	12 Preps
DS0348	Viral RNA Extraction Cartridges	12 nos.
DS0337	1 ml tip	12 nos.
PW1139	Collection Tubes, Polypropylene (2.0 ml)	30 nos.
DS0192	Carrier RNA	0.2 mg
DS0042	Elution Solution (RNase- Free Water)	400 µl
DS0037	RNA Lysis Solution (HRL)	8 ml
DBCA021	Columns for Insta NX	12 nos

**Intended Use**

Recommended for isolation of Viral RNA from various samples like fresh and frozen plasma, serum, nasopharyngeal swab, oropharyngeal swab, sputum, BAL and other body fluids.

**Introduction**

This kit provides a fastest and easiest way to purify viral RNA for reliable use in amplification technologies. Viral RNA can be purified from plasma (treated with anticoagulant EDTA), serum, and other body fluids. Samples may be fresh or frozen, but if frozen, should not be thawed more than once. Repeated freeze–thawing of plasma samples will lead to reduced viral titers and should be avoided for optimal sensitivity. The Super- S columns have a high binding capacity and high quality RNA is obtained from various samples.

**Insta NX® Viral RNA Purification Kit**

This kit carries out efficient extraction of viral RNA from wide range of viral strains like Dengue, Chikungunya and viral pathogens of animals. Sample is first lysed under the highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. When Carrier RNA is added to Elution Solution (RNase- Free Water), it improves the binding of viral RNA to the Super- S Column especially in the case of low-titer samples, and limits possible degradation of the viral RNA due to any residual RNase activity.

The salient features of this kit are as follows:

- Cross- Contamination free
- 12 samples can be processed at a time.
- Prefilled reagent cartridge
- High purity, high yield, as good as manual.

**Elution**

The yield of RNA depends on the sample type and the number of cells in the sample. A single elution with 50 µl of Elution Solution will provide sufficient RNA to carry out multiple amplification reactions. The eluted RNA is suitable for direct use in PCR.

## Concentration, yield and purity of RNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use Elution Solution to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 40 µg/ml of RNA. The  $A_{260}-A_{320}/A_{280}-A_{320}$  ratio should be 1.8–2.1. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. RNA purified by Insta NX® Viral RNA Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of RNA sample (µg/ml) =  $40 \times A_{260} \times \text{dilution factor}$ .

## Storage

Store the Insta NX® Viral RNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 4 months. Store the Carrier RNA (DS0192) at -20°C temperature on receipt. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

## Materials needed but not provided

- Insta NX® (LA1056)
- Vortex Mixer
- Micropipettes (LA617, LA613) and Tips (LA974)

## General Preparation Instructions

- **IMPORTANT: Please go through the instruction manual before starting the experiment.**
- **Preparation of Carrier RNA**

Number of Preps	Carrier RNA	Elution Buffer (RNase free water)
12	0.2 mg	200 µl

Dissolve Carrier RNA thoroughly by pipetting. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

NOTE: Concentration of Carrier RNA to be used is 10µg/ml

**Calculate the volume of Carrier RNA –Lysis Solution (HRL) as follows:**

$$a \times 0.56 \text{ ml} = b \text{ ml}$$

$$b \text{ ml} \times 10 \text{ µl/ml} = c \text{ µl}$$

where, **a** = number of samples to be processed

**b** = volume of Lysis Solution (HRL) to be added for 'a' number of samples

**c** = volume of Carrier RNA to be added to Lysis Buffer (HRL)

eg: for 2 number of samples, add 1.12 ml of Lysis Solution (HRL) and 11.2 µl of Carrier RNA

## Specimen Handling and Collection

Collect plasma, serum or other body fluids in a sterile container. Thaw the samples on ice before use. Repeated freeze- thaw of samples should be avoided.

## Types of Specimen

Clinical samples: Plasma, serum and other body fluids

### Procedure (For Non- Barcode Mode)

1. Turn on Insta NX® instrument. Allow the instrument to initialize.
2. Once the instrument is ready, select “**Login**” on the instrument screen.

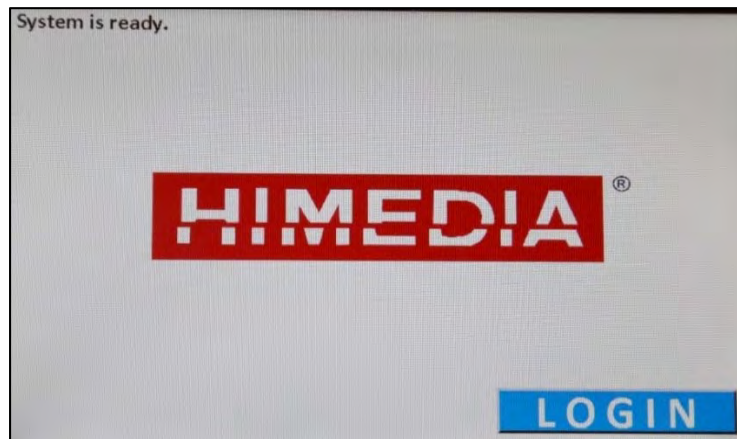


Fig. 1

3. Enter the **Password** to login.

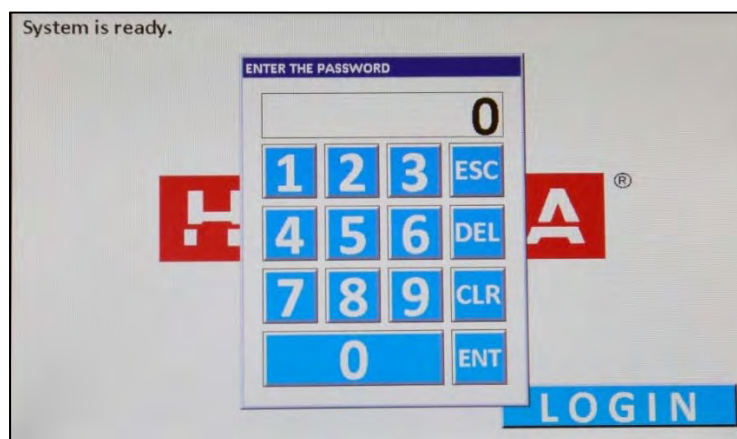


Fig. 2

4. On the Home screen, select “**Start Purification**”

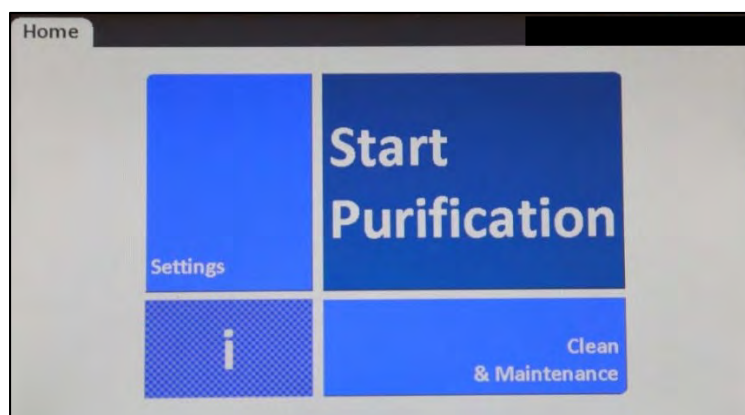


Fig. 3

5. On the **Product Type** screen, select the type of purification to be carried out.



Fig. 4

6. On the Setting screen
  - a. Select Elution volume: 50/ 100/150 or 200µl
  - b. Select Kit Name (type of extraction procedure to be performed).

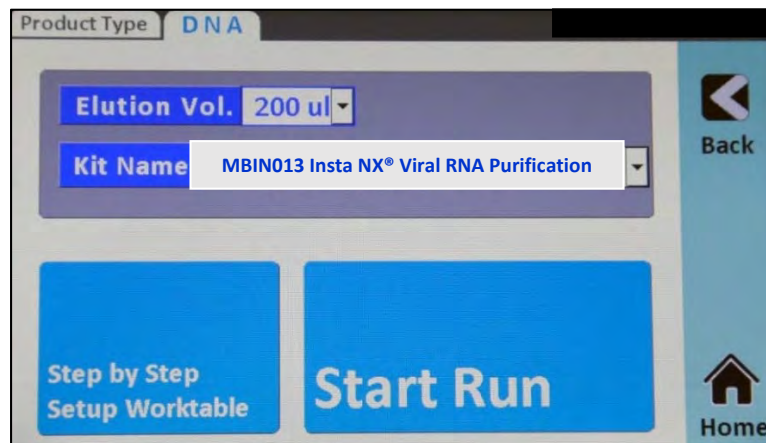


Fig. 5

7. Lift open the front door and take out the 12-in-1 rack for preparation.  
**NOTE:** You can follow **Step by Step Setup worktable** for preparation of 12-in-1 rack
8. Place the Viral RNA Extraction Cartridges (DS0348) on 12-in-1 rack as shown in the below figure.

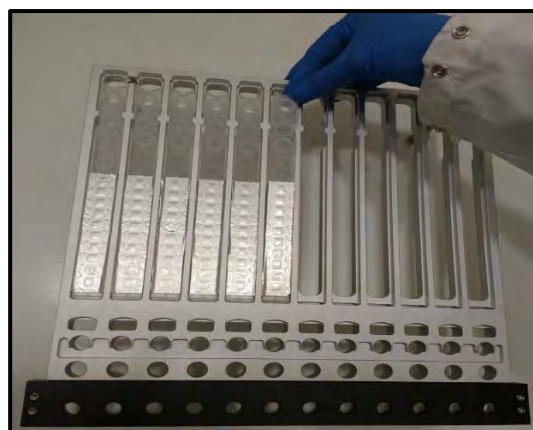


Fig. 6

9. Place the columns (DBCA021) on the cartridge as shown below:

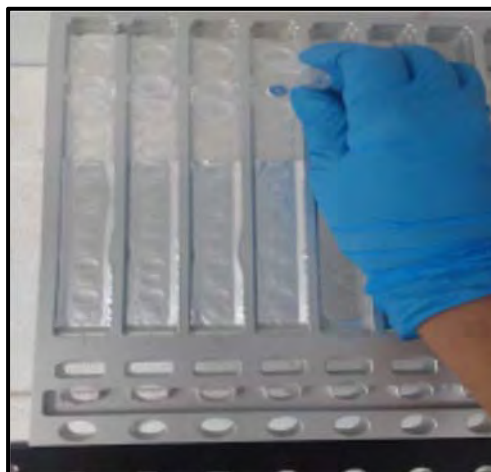


Fig. 7

10. Place 1ml tip (DS0337) set onto the 12-in-1 rack.

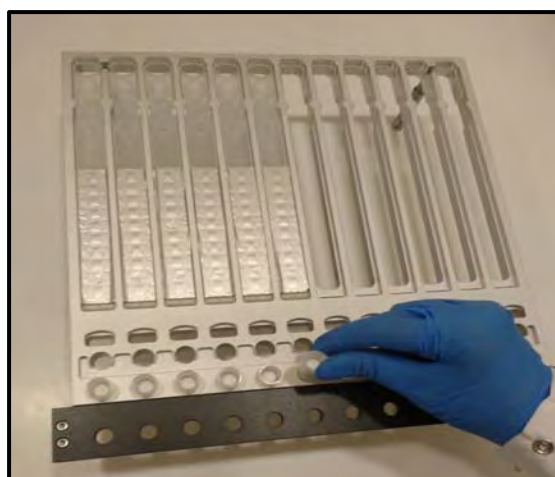


Fig. 8

11. Place Collection tube, Polypropylene (PW1139) on 12-in-1 rack and close the metal lid.



Fig. 9



12. Place the 12-in-1 rack into the instrument as shown in the below fig. and secure the rack in position by two lock plates aside the worktable.

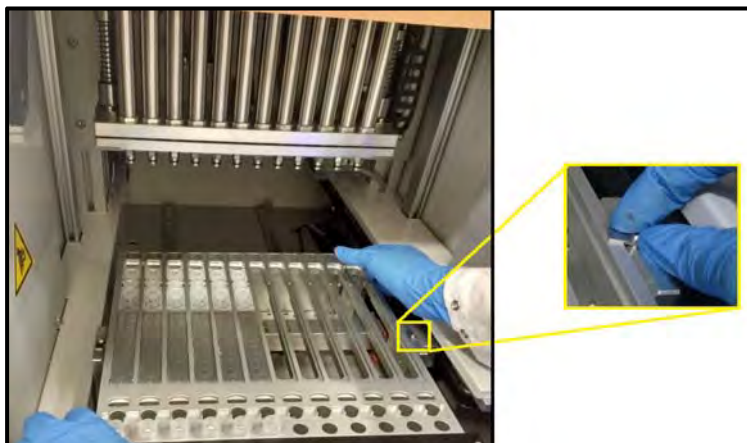


Fig. 10

13. Prepare sample with proper Pre-treatment.

**Sample Pre-treatment procedure**

- Add 140  $\mu$ l of cell free sample like serum, plasma or body fluid, nasopharyngeal swab, oropharyngeal swab, sputum, BAL to Collection Tube, Polypropylene (2.0 ml).
- Add 560  $\mu$ l of RNA Lysis Solution (HRL) - Carrier RNA to the sample.
- Incubate for 10 minutes at room temperature (15-25°C) then proceed with step 14.

14. Place the Collection tube containing sample onto the 12-in-1 rack.

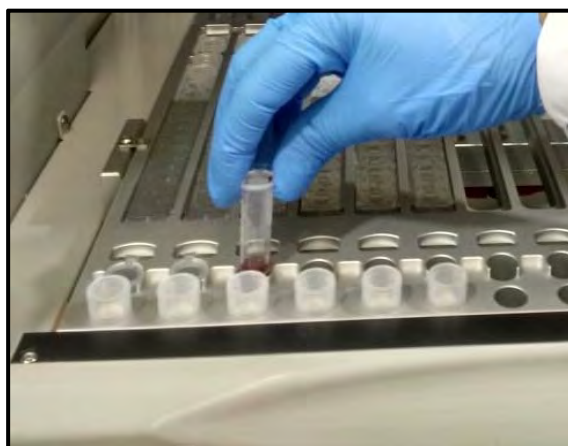


Fig. 10

15. Close the front door. Click **Start Run**.

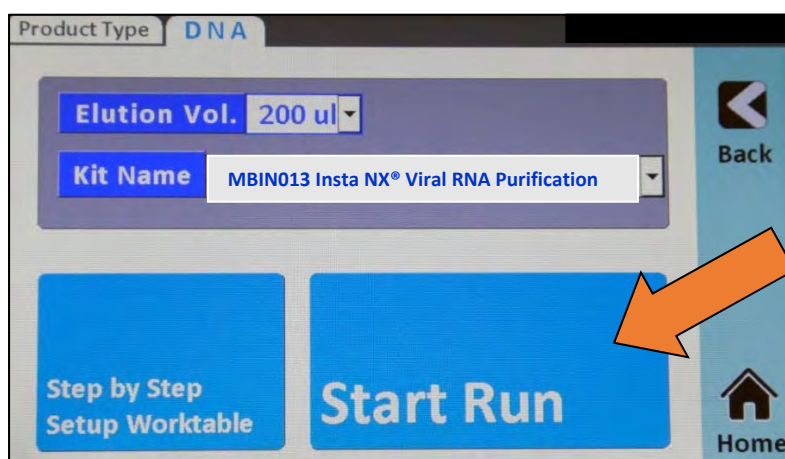


Fig. 11

16. Following screen will appear while the run is going on.

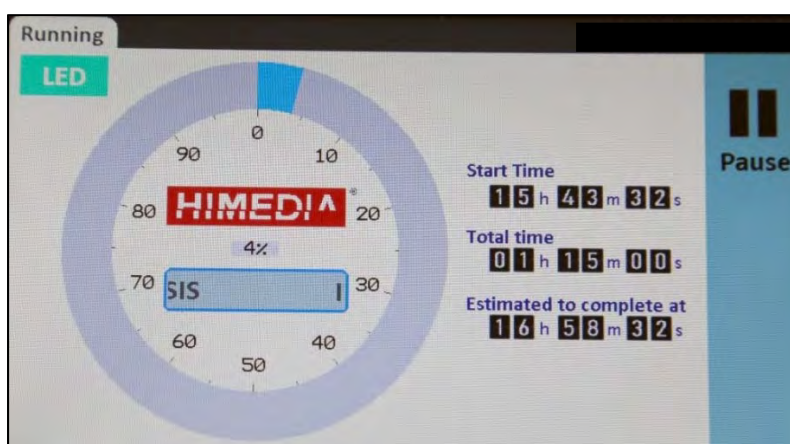


Fig. 12

17. After the run is completed, the screen will look like the below figure:

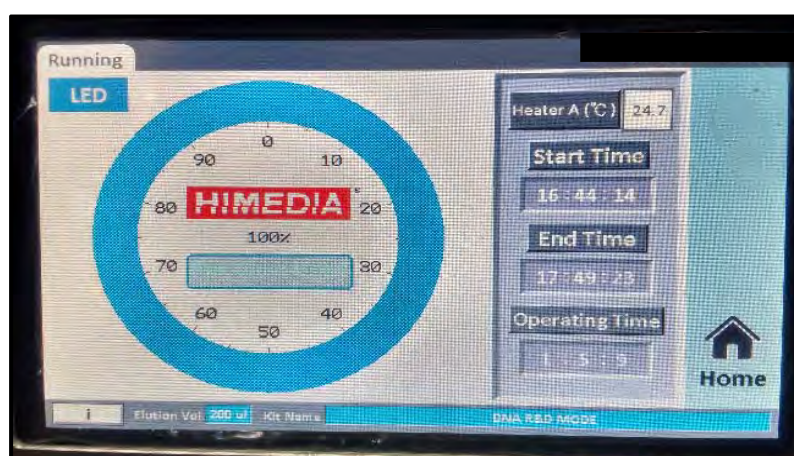


Fig. 13

18. Lift the instrument lid and take out the 12-in-1 rack. Remove the elution tubes and discard all other plastic wares.

19. The eluted RNA can be further used for PCR amplification.



Fig. a: Insta Q 96 PLUS

Fig. b: Wee Machine

Representative image of PCR set up in HiMedia's PCR Machines

**Storage of the eluate with purified RNA:** The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

#### Warning

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

#### Performance and Evaluation

Each lot of HiMedia's Insta NX® Viral RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

#### Quality Control

Type of sample	PCR amplification
Dengue serum sample	Observed

#### Troubleshooting Guide

Sr.No.	Problem	Possible Cause	Solution
1.	Poor / Lower yield of RNA	Sample is too old	Yield of RNA varies from sample to sample. It is necessary to use the starting sample as per the protocol.

Please refer disclaimer Overlay.



## Safety Information

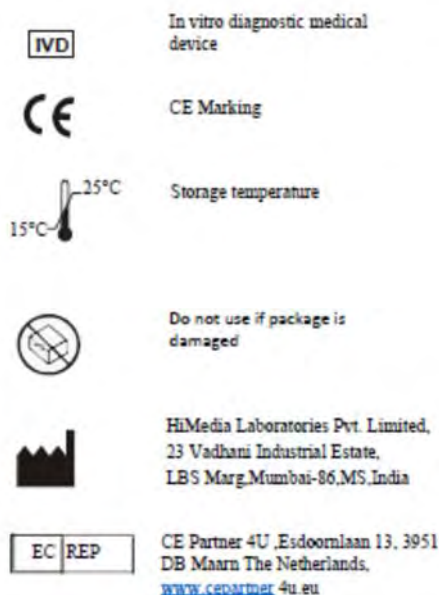
Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

## Disposal

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

## Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to [cs@ewcdiagnostics.com](mailto:cs@ewcdiagnostics.com).



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### Disclaimer :

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