Standard Operation Procedure

Viral RNA Extraction using Pre- filled Plates for Insta NX® Mag96 (27 mins protocol)

Introduction:

Nucleic acids, DNA and RNA, hold valuable biological information. Extracting this valuable information plays a pivotal role in multiple areas of life science and healthcare. RNA plays an essential role in various biological processes involving gene regulation and gene expression. Molecular diagnostics using RNA based analysis have a wide range of applications across various areas of molecular diagnostics, as they serve as biomarkers for disease diagnosis and prognosis.

Storage condition:

The viral RNA extraction kit can be kept at room temperature.

Kit contents (per kit):

Plate name	Contents	Storage	Quantity
Plate 3 (P3)	Lysis/Binding buffer	RT	1 no
Plate 5 (P5)	Wash Buffer I	RT	1 no
Plate 6 (P6)	Wash Buffer II + CamBead SI solution	RT	1 no
Plate 7 (P7)	—------	RT	1 no
Plate 8 (P8)	Elution Buffer	RT	1 no
96-well Magnetic Tip Comb	—-------	RT	1 no
Proteinase K(Lyophilized)	20 mg	-20°C (upon reconstitution)	1 no
Carrier RNA(lyophilized)	1 mg	-20°C (upon reconstitution)	1 no
Proteinase K diluent	1 ml	RT	1 no
Carrier RNA diluent	2 ml	RT	1 no

How to start:

Items required by the user but not provided in the kit:

Equipment:

Insta NX® Mag96

Recommended Sample volume for starting:

A starting sample volume of 200 µL (Nasopharyngeal/Oropharyngeal swabs in VTM) is recommended for viral RNA extraction.

Preparation of working solutions

1. Add 1.5 mL of Nuclease free water provided in the kit to 1 mg of carrier RNA and resuspend it thoroughly. After resuspension the **Carrier RNA** should be stored at -20°C.

2. Reconstitute 20 mg of lyophilized **Proteinase K** powder by adding 1 mL of Proteinase K diluent. After reconstitution the Proteinase K should be stored at -20°C.

Plate constituents:

Buffer	Volume of buffer per well	Plate no
Lysis Buffer Sample Proteinase K solution Carrier RNA solution	500 µL 200 µL 10 µL 10 µL	P3
Wah Buffer I	500 µL	P4
Wash Buffer II + CamBeads SI solution	500 µL	P5
Elution buffer	60 µL	P8

Protocol:

1. Place the VTM tube with the sample on a shaker for ~20min to allow release of sample from the swab into the solution. Vortex the tube thoroughly for 30 seconds, before adding the sample.

   Note: Shake all the Plates gently once, before using.

2. Select program **MB615MA** from the Home screen.

3. Take 200µl of sample from the VTM tube and add the sample in each well of **Plate 3 (P3)**. Sequentially, add 10 µL Proteinase K solution and 10 µL Carrier RNA solution to each well containing the sample.
4. Select plate position 3 on the screen.
5. Open the door of the Insta NX® Mag96 machine.
6. Place the plate on 3rd position onto the machine after adding the above mentioned solutions.
7. Select plate position 5 on the screen.
8. Place **Plate 5 (P5)** on 5th position onto the machine.
9. Select plate position 6 on the screen.
10. Place **Plate 6 (P6)** on 6th position onto the machine.
11. Select plate position 7 on the screen.
12. Insert Magnetic Tip comb for Insta NX® Mag96 and place the **Plate 7 (P7)** on 7th position onto the machine.
13. Select plate position 8 on the screen. Place the **Plate 8 (P8)** on 8th position onto the machine.
14. Close the door of the machine.
15. Click on the RUN button on the home screen for Program **MB615MA.**
16. On completion of the protocol, store Plate 8 (P8) that contains pure eluted RNA. Discard all other plates

Note: Store the eluted RNA at -80°C.