Standard Operating Procedure

Viral RNA extraction using Cambeads Si on KINGFISHER FLEX(96 reactions)

Background

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)

Kit contents	Component	Quantity provided per kit
Plate 1	Lysis/Binding Buffer	50ml
Plate 2	Wash Buffer I 50ml	
Plate 3	Wash Buffer II + Mag bead solution	50ml
Plate 4	Elution buffer	6ml
96 Tip Comb	1 no	
Proteinase-K	1no	20mg
Carrier RNA	1no	1mg
Proteinase-K Diluent	1no	1ml
Carrier RNA Diluent	1no	2ml

How to start

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

Equipment:

Thermofisher Kingfisher Flex system

Recommended Sample volume for starting

A starting sample volume of 200 μL is recommended for viral RNA extraction.



Preparation of working solutions

- 1. Add 1.5 mL of Carrier RNA diluent provided in the kit to 1 mg of carrier RNA and resuspend it thoroughly. After resuspension, the Carrier RNA is stored at -20°C.
- 2. Reconstitute 20 mg of lyophilized **Proteinase K** powder by adding 1mL of Proteinase K diluent. After reconstitution the Proteinase K is stored at -20°C.

Plate Constituents

Buffer	Plate Number	Vol of buffer per well
Lysis Buffer		500μL
Sample		200 μL
Proteinase K (reconstituted)	Plate 1	10 μL
Carrier RNA (reconstituted)		10 μL
Wash Buffer I	Plate 2	500 μL
Wash Buffer II+ Mag bead solution	Plate 3	500 μL
Elution plate	Plate 4	60 μL
96 tip comb		

RNA extraction from Nasopharyngeal / Oropharyngeal swabs using EXM-6000 (96 reactions)

Protocol

- 1. Vortex the VTM tube with the sample for 30 seconds to allow the release of the sample from the swab into the solution.
- 2. Take 200 μ l of sample from the VTM tube and add the sample into all wells of Plate 1 containing Lysis and Binding buffer. Add 10 μ L reconstituted Proteinase K and 10 μ L reconstituted Carrier RNA to each well containing the sample.
- 3. Keep the plate 1 onto deck position- 1.
- 4. Put 9ó Pin Deep Well Tip Comb into plate 2, slot into deck position-2 on the Purifier 9ó
- 5. Immediately load the remaining plates onto the Instrument
- 6. Select KF_ViralNA_Flex96 program on the Kingfisher Flex.
- 7. At the end of the run, remove the plate-4 from the Instrument, then transfer the solution to the final tubes/plate.

Note:- The purified nucleic acid is ready for immediate use. Alternatively, store the plate at -20 degrees celsius for long-term storage.

