# Standard Operation Procedure

## VIRAL RNA EXTRACTION USING ZYBIO EXM-3000

### 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):**

| Contents | Quantity | Storage |
|---|---|---|
| Lysis Buffer | 64 mL | RT |
| Mag bead solution | 20ml | RT |
| Wash Buffer | 64ml | RT |
| Elution buffer | 6ml | RT |
| Proteinase K (lyophilized) | 80 mg | -20°C (upon reconstitution) |
| Proteinase K diluent | 4mL | RT |
| Carrier RNA (lyophilized) | 4 mg | -20°C (upon reconstitution) |
| Carrier RNA diluent | 4 mL | RT |

**How to start:**

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

Equipment:

1. Zybio EXM-3000

**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 mL of Nuclease free water 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 1 mL of Proteinase K diluent. After reconstitution the Proteinase K is stored at -20°C.

**RNA extraction from Nasopharyngeal / Oropharyngeal swabs using Zybio EXM 3000**

**Plate constituents:**

| Contents | Well no | Volume(ul) per well |
|---|---|---|
| Lysis Buffer<br>Sample<br>Proteinase K (reconstituted)<br>Carrier RNA (reconstituted) | 1 and 7 | 500<br>200<br>10<br>10 |
| Mag bead solution | 2 and 8 | 150 |
| Wash Buffer | 3 and 9 | 500 |
| Elution buffer | 6 and 12 | 40 |

**Protocol:**

1. Place the VTM tube with the sample on a vortex to allow mixing of samples thoroughly for 30 seconds, before adding it to the wells. Fill in the plate with the respective buffers according to the above mentioned table.

2. Take 200µl of sample from the VTM tube and add the sample into respective wells of column 1 and column 7, containing the lysis buffer. Add 10 µL Proteinase K and 10 µL Carrier RNA to each well containing the sample.

3. Fit in the magnetic sleeves and the reaction plate firmly on the Zybio platform. Run Protocol **Zybio-B-200**.

4. On completion of the protocol, collect the eluted sample from wells of column 6 and 12 in a clean RNAse free 1.5ml microcentrifuge tube. Store the eluted RNA at -80°C.