STANDARD OPERATING PROCEDURE

SOP # 1 Page: 1 of 3	Version 1.1 Date: 07/08/2017 Revise by: 07/08/2017	Prepared By: **Dr Joyanta Modak** Approved By: **Dr Vijay Dhanasekaran**

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SOP 1: Virus RNA isolation using QIAamp Viral RNA mini kit

1. Introduction

This SOP describes the use of QIAamp Viral RNA mini kit (QIAGEN) to isolate RNA from sample containing viruses.

2. Scope

This procedure serves as a guide to laboratory personnel engaged in viral RNA purification using the QIAamp® Viral RNA Mini Kit by QIAGEN.

3. Safety

All lab workers must:

a. Wear appropriate personal protective equipment (PPE) (Nitrile gloves, lab coat, closed-toed shoes, and safety glasses when specified by risk assessment) when carrying out the procedure.
b. If procedure is performed on reassortant viruses, please wear an N95 mask for additional personal protection.
c. Carry out the procedure in the appropriate setting, i.e. tissue culture room.
d. Read and understand the recommendations in this SOP to ensure uniformity in practice.
e. New staff or students should seek supervision from a senior lab co-worker during first attempt of the procedure.

4. Definition

Viral RNA purification: To extract purified viral RNA from samples plasma, serum, cell-free body fluids or cell-culture supernatants.

5. Procedures

5.1 Materials Needed

a. Virus-infected cell-culture supernatant
b. QIAamp® Viral RNA Kit (250) (QIAGEN Cat# 52906), which includes:
   i) QIAamp® Mini spin columns
   ii) Collection tubes (2 mL)
   iii) Buffer AVL

iv) Buffer AW1
v) Buffer AW2
vi) Buffer AVE
vii) Carrier RNA (poly A)

c. Ethanol (96 – 100%)
d. 1.5 mL micro-centrifuge tubes (Axygen Cat# MCT-150-C)
e. ART 200 and ART 1000XL aerosol barrier pipette tips (Molecular Bio-Products Cat# 2069-05-HR and 2179-05-HR)
f. P100 and P1000 single-channel pipettes (Eppendorf Cat # 3111000.149 and 31111000.165)
g. Micro-centrifuge
h. Freezer -20°C
i. Freezer -80°C

5.2 Reagent Preparation

To prepare carrier-RNA-containing Buffer AVL, carry out the following in accordance:

a. Add 310 µL of Buffer AVE to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µL.
b. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at -20°C. Do not freeze-thaw the aliquots of carrier RNA more than 3 times.
c. For each sample, add 1 volume of carrier RNA-AVE to 100 volumes of Buffer AVL. (i.e. 5.6 µL of carrier RNA-AVE to 0.56 mL of Buffer AVL for 1 sample)

To prepare Buffer AW1 and Buffer AW2, add the appropriate amount of ethanol (96 – 100%) as indicated on the bottle.

5.3 Purification of Viral RNA using Spin Columns

a. Pipette 560 µL of prepared Buffer AVL containing carrier RNA into a 1.5 mL microcentrifuge tube. (If the sample volume is larger than 140 µL, increase the amount of Buffer AVL-carrier RNA proportionally.)
b. Add 140 µL cell-culture supernatant to the Buffer AVL-carrier RNA in the micocentrifuge tube. Mix by pulse-vortexing for 15 s.

c. Incubate at room temperature (15 – 25°C) for 10 min.
d. Briefly centrifuge the tube to remove drops from the inside of the lid.
e. Add 560 µL of ethanol (96 – 100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove drops from inside the lid. (If the sample volume is greater than 140 µL, increase the amount of ethanol proportionally.)
f. Carefully apply 630 µL of the solution from the previous step to the QIAamp Mini column (in a 2 mL collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini column into a clean 2 mL collection tube, and discard the tube containing the filtrate.
g. Carefully open the QIAamp Mini column, and repeat the previous step.
h. Carefully open the QIAamp Mini column, and add 500 µL of Buffer AW1. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 mL collection tube (provided), and discard the tube containing the filtrate.
i. Carefully open the QIAamp Mini colum, and add 500 µL of Buffer AW2. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
j. (Opional) Place the QIAamp Mini column in a new 2 mL collection tube (not provided), and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
k. Place the QIAamp Mini column in a clean 1.5 mL microcentrifuge tube (not provided). Discard the old collecion tube containing the filtrate. Carefully open the QIAamp Mini column and add 60 µL of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6000 x g (8000 rpm) for 1 min.
l. Store the purified viral RNA at -20°C or -70°C.

1.0 RECORDS

Nil

2.0 APPENDIXES

Nil

3.0 REFERENCES

QIAGEN, 2010. *QIAamp® Viral RNA Mini Handbook.* **Third Edition**.