BECKMAN COULTER Life Sciences

Viral RNA extraction with RNAdvance Viral XP

Please reference part number on reagent bottles for product safety information (SDS) on Beckman.com. Do not use reagents beyond the expiration date on the bottle. Use Good Laboratory Practices (GLP) when handling reagents.

Researchers who want to extract viral RNA from swab samples should use this protocol. Samples should be handled as if capable of transmitting infection and disposed of with proper precautions.

Purpose

The following protocol allows for the isolation of viral RNA from 200 µL of swab collection media in 1.5 mL tube or 96-well plate formats. Purification begins with lysis of the viral capsid. Following lysis, the RNA is immobilized onto the magnetic particles allowing separation from contaminants using a magnetic field. The contaminants are then rinsed away using a simple wash procedure.

Materials Required

Material	Part Number	Supplier
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	AM9932	ThermoFisher Scientific
1.5 mL Microcentrifuge Tubes	0030119401	Eppendorf
SPRIStand Magnetic 6 Tube Stand	A29182	Beckman Coulter
RNAdvance Viral XP	C58581	Beckman Coulter

Protocol

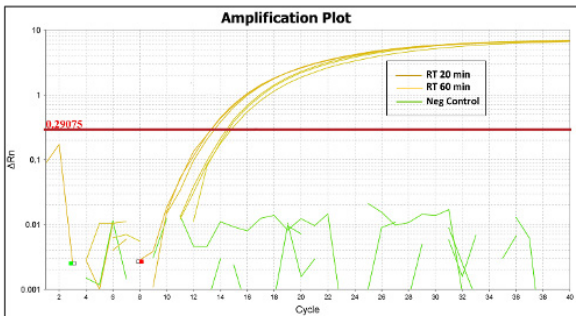
1. Sample Preparation
    A. Vortex the samples **10 sec** at **maximum speed** on a vortex to resuspend the sample
    B. Briefly centrifuge the samples to collect the all liquid on the tube cap
2. Lysis
    A. Transfer **200 µL** of **sample** to 1.5mL microcentrifuge tube or 96-well plate
    B. Add **150 µL** of **Lysis buffer (LBF)** to the sample
    C. **Mix** by pipetting up and down 10 times or until thoroughly mixed.
    D. **Incubate** sample at RT for **20 min**
3. Bind
    A. Vortex the bottle of **RNAClean XP** to fully resuspend the beads
    B. Add **350 µL** of **RNAClean** to the sample
    C. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    D. Incubate the plate for **5 minutes** at **room temperature**
    E. Place the sample on a **magnet** for **10 minutes** (or until the supernatant is clear)
    F. Remove and discard the supernatant without disrupting the beads
    G. Remove the sample from the magnet

4. Wash
    A. Add **400 µL** of freshly prepared **70% ethanol** to the sample
    B. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    C. Place the sample on the **magnet** for **2 minutes** (or until the supernatant is clear)
    D. Remove and discard the supernatant without disrupting the beads
    E. Repeat steps 4.A-4.D for a total of **2 ethanol washes**
    F. Place the sample on a **magnet** to dry for **1 minute** (or until no liquid is visible, may consider using P200 to remove visible liquid while the sample is on a magnet)
    G. Remove the sample from the magnet
5. Elute
    A. Add **40 µL** of **nuclease-free** water to the sample
    B. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    C. Incubate the sample for **5 minutes** at **room temperature**
    D. Place the sample on a **magnet** for **2 minutes** (or until the supernatant is clear)
    E. Remove and **Save** the supernatant without disrupting the beads

Example Data

Swabs dipped in coronavirus strain 229E culture fluid (ZeptoMetrix Cat# 0810229CF) were resuspended in 200 µL of PBS. RNA was isolated using RNAdvance Viral XP kit. After extraction, 5.5 µL of RNA was used in a 10 µL reaction for qRT-PCR using Reliance One-Step Multiplex RT-qPCR Supermix (Bio Rad Cat#: 12010220). Fluorescence intensity was monitored at each PCR cycle using the Applied Biosystems QuantStudio 6 Flex real-time PCR system (ThermoFisher) for 40 cycles. Each of the conditions were performed in triplicate. RT and ND indicate room temperature and not determined, respectively. The following primers and probe (ThermoFisher) were used: 229E-FP: 5′-TTCCGACGTGCTCGAACTTT-3′, 229E-RP: 5′-CCAACACGGTTGTGACAGTGA-3′, and 229E-TP: FAM-5′-TCCTGAGGT CAATGCA-3′-NFQ-MGB; nt 506 to 521.

qRT-PCR of RNA extracted from Coronavirus 229E using RNAdvance Viral XP



Two lysis conditions (RT for 20 min and RT for 60 min) were assessed. A Ct shift of 1.12 is seen at 60 min vs. 20 min. The data indicates that 20 minutes is sufficient for the release and isolation of RNA. However, it is critical to consider that this data does not confirm that viral inactivation occurs in either of these lysis conditions.

Sample Input	Lysis Conditions	Ct values	Std Dev
229E	RT / 20 min	13.45	.155
229E	RT / 60 min	14.57	.195
Water (-control)	RT / 60 min	ND	ND

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