BECKMAN COULTER Life Sciences

Viral Nucleic Acid extraction from Swabs using RNAdvance Viral

Please reference the current RNAdvance Blood Protocol for product and safety information (Product Number: A35604, A35603).

Researchers who want to extract nucleic acids from an RNA virus or a DNA virus should use this protocol.

Purpose

The extraction of nucleic acids from samples containing viral DNA or RNA is important for both pathogen detection and microbiome discovery. The method presented here is a modified RNAdvance Blood protocol that can extract both RNA and DNA from viral samples collected on from swab samples. The reagent volumes were modified for 200 µL swab collection media. Different input volumes will require reagent volume modification. This protocol is for swab samples collected and stored according to the manufacturer's instructions.

Additional Materials Required

Material	Part Number	Supplier
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
100 % Isopropanol (Molecular Grade)	AB07015-01000	AmericanBio
Nuclease-free water (Molecular Grade)	AM9932	ThermoFisher Scientific
1.5 mL Microcentrifuge Tubes	0030119401	Eppendorf
SPRIStand Magnetic 6 Tube Stand	A29182	Beckman Coulter Life Sciences
RNAdvance Viral	C57955, C57956	Beckman Coulter Life Sciences

*Materials above are suggested; equivalent materials can be used.

Protocol

1. **Sample Preparation**
    A. Vortex the sample for **2 min** 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 **swab collection media** to 1.5 mL microcentrifuge tube
        I. Add **10 µL** of **Proteinase K (PK)** to tube
            a. To prepare Proteinase K+ PK Buffer:
                i. For smaller kit (C57955), add **1.2 mL** of **PK Buffer** to tube of **Proteinase K**
                ii. For large kit (C57956), add **10 mL** of **PK Buffer** to tube of **Proteinase K**
        II. Add **150 µL** of **Lysis LBF** to tube
    B. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    C. **Incubate** the tube for **20 minutes** at **room temperature**

3. **Bind**
    A. Vortex the bottle of **Bind BBD** to fully resuspend the beads
    B. Prepare **BBD/isopropanol** solution
        I. Add **200 µL** of **isopropanol** to a mixing vessel
        II. Add **5 µL** of **BBD** to the mixing vessel
    C. Add **205 µL** of **BBD/isopropanol** solution to the sample
    D. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    E. Incubate the tube for **5 minutes** at **room temperature**
    F. Place the tube on a **magnet, SPRIStand Magnetic 6 Tube Stand,** for **10 minutes** (or until supernatant is clear)
    G. Remove and discard the supernatant without disrupting the beads
    H. Remove the tube from the magnet
4. **Wash**
    A. Add **400 µL** of **Wash WBE** to the sample
        I. To prepare **Wash WBE**:
            a. For small kit (C57955), add **30 mL** of **100% Isopropanol** to **Wash WBE** (C42160)
            b. For large kit (C57956), add **225 mL** of **100% Isopropanol** to **Wash WBE** (C42172)
    B. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    C. Place the tube on a **magnet** for **5 minutes** (or until supernatant is clear)
    D. Remove and discard the supernatant without disrupting the beads
    E. While tube is on the magnet, add **400 µL** of **70% ethanol** to the plate
    F. Leave the tube on a **magnet** for **2 minutes** (or until supernatant is clear)
    G. Remove and discard the supernatant without disrupting the beads
    H. Repeat steps 4.E-4.G for a total of **2 washes**
    I. Place the tube on a **magnet** to dry for **1 minute** (or until no liquid is visible)
    J. Remove the tube from the magnet
5. **Elute**
    A. Add **40 µL** of nuclease free water to the plate
    B. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    C. Incubate the plate for **5 minutes** at room temperature
    D. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
    E. Remove and **Save** the supernatant without disrupting the beads

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