



# 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  $\mu$ L 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  $\mu$ L** of **swab collection media** to 1.5 mL microcentrifuge tube
  - I. Add **10  $\mu$ L** 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  $\mu$ L** 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, SPRISand 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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