



## Viral Nucleic Acid extraction using RNAdvance Blood

Please reference the current RNAdvance Blood Protocol for product information (Product Number: A35604, A35603, A35605).

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.

### 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
1.2 mL 96-well plate	AB1127	ThermoFisher Scientific
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
RNAdvance Blood Kit	A35604, A35603, A35605	Beckman

### Protocol

#### 1. Lysis

- a. Transfer **200 µL** of **sample** to 1.2 mL 96-well plate
  - i. Add **10 µL** of **Proteinase K (PK)** to plate
  - ii. Add **150 µL** of **Lysis** to plate
- b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
- c. **Incubate** the plate for **20 minutes** at **room temperature**

#### 2. Bind

- a. Vortex the bottle of **Bind 1** to fully resuspend the beads
- b. Prepare **Bind 1/isopropanol** solution
  - i. Add **200 µL** of **isopropanol** to a mixing vessel
  - ii. Add **5 µL** of **Bind 1** to the mixing vessel
- c. Add **205 µL** of **Bind 1/isopropanol** solution to the sample
- d. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
- e. Incubate the plate for **5 minutes** at **room temperature**
- f. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
- g. Remove and discard the supernatant without disrupting the beads
- h. Remove the plate from the magnet

### 3. Wash

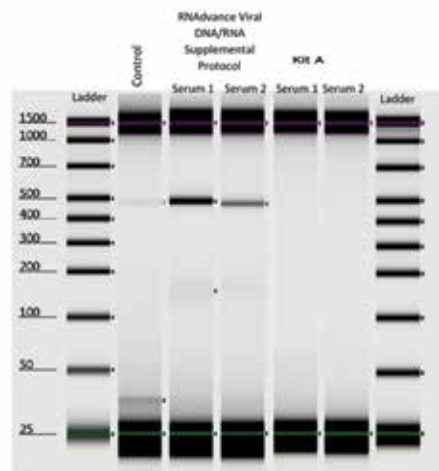
- a. Add **400 µL** of **Wash** to the sample
- b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
- c. Place the plate on a **magnet** for **5 minutes** (or until supernatant is clear)
- d. Remove and discard the supernatant without disrupting the beads
- e. Remove the plate from the magnet
- f. Add **400 µL** of **70% ethanol** to the plate
- g. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
- h. Place the plate on a **magnet** for **1 minute** (or until supernatant is clear)
- i. Remove and discard the supernatant without disrupting the beads
- j. Remove the plate from the magnet
- k. Repeat steps 3.f-3.j for a total of **2 washes**
- l. Place the plate on a **magnet** to dry for **1 minute** (or until no liquid is visible)
- m. Remove the plate from the magnet

### 4. Elute

- a. Add **20 µL** of nuclease free water to the plate
- b. Incubate the plate for **5 minutes** at room temperature
- c. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
- d. Remove and **Save** the supernatant without disrupting the beads

## Example Data

To test for viral DNA extraction from HBV, six HBV positive sera were extracted using 2 different extraction kits, including the supplemental protocol presented above. The extraction was proceeded with a one-step PCR amplification and visualized using an Agilent 4200 Tape Station (Agilent Technologies) (Figure 1).



**Figure 1.** DNA was extracted from HBV positive serum using the presented RNAAdvance Viral DNA/RNA supplemental protocol and another commercially available kit. Following extraction a one-step PCR amplification was performed to amplify a 500 bp fragment. Lanes 2, 3 and 4 showed amplification of this product indicating a positive result for extraction of HBV DNA

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