BECKMAN COULTER
Life Sciences

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).

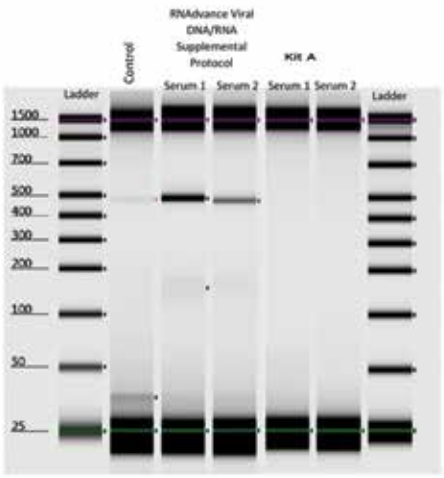

Figure 1. DNA was extracted from HBV positive serum using the presented RNAdvance Viral DNA/RNA supplemental protocol and another commercially available kit. Following extraction a one-step PCR amplification was performed to amplify a 500 bp fragment. Lanes 2, 3 and 4 showed amplification of this product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

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