

Total RNA Isolation: 100 μ l Viral Sample from Cultured-Cell Supernatant Containing Virus

AquaPure[®] RNA Isolation Kit

Catalog #732-6370

Yield: Dependent on sample and RNA expression levels

Method

Sample Collection and Handling

Samples may be fresh or frozen. Collect fresh virus and isolate RNA as quickly as possible; store at 1–8°C for not more than 24 hr. For long-term storage, store at –70 to –80°C.

Cell Lysis

1. Add 500 μ l cell lysis solution to a 1.5 ml microfuge tube that contains 100 μ l virus from cultured-cell supernatant.
2. Cap the tube and vortex for 5 sec to mix thoroughly.
3. Heat sample to 65°C for 5 min to complete lysis. Cool sample to room temperature. (This step is optional when testing for certain viruses such as hepatitis C.)

Protein-DNA Precipitation

1. Add 200 μ l protein-DNA precipitation solution to the viral cell lysate.
2. Invert capped tube gently 10 times and place tube into an ice bath for 5 min.
3. Centrifuge at 13,000–16,000 x g for 3 min. The precipitated proteins and DNA will form a tight pellet.

RNA Precipitation

1. Leaving behind the precipitated protein-DNA pellet, pour the supernatant containing the RNA into a clean 1.5 ml microfuge tube containing 600 μ l 100% isopropanol (2-propanol). If RNA yield is expected to be low (<1 mg), add glycogen as a carrier to the isopropanol. We recommend adding 1 μ l of glycogen solution (20 mg/ml) per 600 μ l isopropanol.
2. Cap the tube and mix the sample by inverting gently 50 times.
3. Centrifuge at 13,000–16,000 x g for 3 min; depending on yield, the RNA may or may not be visible as a small, translucent pellet.

4. Pour off supernatant and drain tube on clean absorbent paper. Add 600 μ l 70% ethanol and invert the capped tube several times to wash the RNA pellet.
5. Centrifuge at 13,000–16,000 x g for 1 min. Carefully pour off the ethanol.
6. Invert and drain the tube on clean absorbent paper and allow to air-dry 10–15 min.

RNA Hydration

1. Add 20 μ l RNA hydration solution (20 μ l will give a concentration of 50 μ g/ml if the total yield is 1 μ g RNA).
2. Allow RNA to rehydrate on ice for at least 30 min. Store purified RNA sample at –70 to –80°C until use.
3. Before use, vortex sample vigorously for 5 sec and pulse-spin. Pipet sample up and down several times to ensure adequate mixing.

Ordering Information

Catalog #	Description
732-6370	AquaPure RNA Isolation Kit, for animal and plant tissues, cultured cells, and gram-negative bacteria, processes up to 100 x 0.5–10 mg animal or plant tissue preps, 100 cultured cell preps (1–2 x 10 ⁶ cells/prep), or 100 x 0.5 ml bacterial cultures per kit

Related Products

732-6371	AquaPure RNA Blood Kit, for human and mammalian whole blood and bone marrow, processes up to 100 x 0.3 ml whole blood samples per kit
732-6343	AquaPure Genomic DNA Isolation Kit, for cultured cells and gram-negative bacteria, processes up to 100 cultured cell preps (1–2 x 10 ⁶ cells/prep), or 100 x 0.5 ml bacterial cultures per kit
732-6343	AquaPure Genomic DNA Tissue Kit, for animal and plant tissues, cultured cells, and gram-negative bacteria, processes up to 100 x 0.5–10 mg animal or plant tissue preps, 100 cultured cell preps (1–2 x 10 ⁶ cells/prep), or 100 x 0.5 ml bacterial cultures per kit
732-6345	AquaPure Genomic DNABlood Kit, for human and mammalian whole blood and bone marrow, processes up to 100 x 0.3 ml whole blood samples per kit