

## Total RNA Isolation from 500 $\mu$ l Body Fluid

### AquaPure® RNA Isolation Kit

Catalog #732-6370

#### Method

##### Cell Lysis

1. Vigorously add 2.5 ml cell lysis solution to an Oak Ridge centrifuge tube (or other tube rated for high speed) containing 500  $\mu$ l body fluid (e.g., cerebrospinal fluid, plasma, saliva, serum, sputum, synovial fluid, urine, whole blood).
2. 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 1 ml protein-DNA precipitation solution to the lysate.
2. Cap tube and invert gently 10 times and place tube into an ice bath for 10 min.
3. Centrifuge at 15,000 x g for 5 min. The precipitated proteins and DNA will form a tight pellet.

##### RNA Precipitation

1. Pour the supernatant containing the RNA (leaving behind the precipitated protein-DNA pellet) into a clean Oak Ridge centrifuge tube (or other tube rated for high speed) containing 3 ml 100% isopropanol (2-propanol). If RNA yields are expected to be low, add a glycogen solution as a carrier to the isopropanol (5  $\mu$ l of 20 mg/ml glycogen per 3 ml isopropanol).
2. Mix the sample by capping and inverting gently 50 times.
3. Centrifuge at 15,000 x g for 5 min; depending on yield, the RNA may or may not be visible as a small, translucent pellet.
4. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 3 ml 70% ethanol, cap the tube, and invert it several times to wash the RNA pellet.
5. Centrifuge at 15,000 x g for 2 min. Carefully pour off the ethanol.
6. Invert and drain the tube on clean absorbent paper and allow sample to air-dry for 15 min.

##### RNA Hydration

1. Add 100  $\mu$ l RNA hydration solution (100  $\mu$ l will give a concentration of 100  $\mu$ g/ml if the total yield is 10  $\mu$ g RNA).
2. Allow RNA to rehydrate for at least 30 min on ice. Alternatively, 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 mix the sample further.

### Ordering Information

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<sup>6</sup> 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

732-6340 AquaPure Genomic DNA Isolation Kit, for cultured cells and gram-negative bacteria, processes up to 100 cultured cell preps (1–2 x 10<sup>6</sup> 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<sup>6</sup> cells/prep), or 100 x 0.5 ml bacterial cultures per kit

732-6345 AquaPure Genomic DNA Blood Kit, for human and mammalian whole blood and bone marrow, processes up to 100 x 0.3 ml whole blood samples per kit