

Isolation of Genomic DNA from Cultured-Cell Supernatant Containing Virus (50 µl Viral Sample)

AquaPure® Genomic DNA Isolation Kit

Catalog #732-6340

Expected yield: 0.1–2.5 µg DNA

Method

Sample Collection and Handling

Samples may be fresh or frozen. Collect virus and isolate genomic DNA as quickly as possible; store at 1–8°C for 24 hr. When cell numbers are low, concentrate samples by centrifugation at 2,000 x g for 10 min. Remove supernatant, leaving behind 50 µl residual liquid to thoroughly resuspend the pellet. Keep sample on ice or store frozen at –70 to –80°C.

Cell Lysis

1. Add 250 µl cell lysis solution to a 1.5 ml microfuge tube that contains 50 µl virus.
2. Incubate at 65°C for 15 min to complete lysis. Or, if maximum yield is required, add 1.5 ml proteinase K solution (20 mg/ml) to the cell lysate; cap the tube and mix by inverting 25 times, then incubate at 55°C for 1 hr to overnight.

RNase Treatment (Optional)

1. Add 1.5 µl RNase A solution to the cell lysate.
2. Mix the sample by inverting the capped tube 25 times and incubate at 37°C for 15–60 min.

Protein Precipitation

1. Cool sample to room temperature.
2. Add 100 µl protein precipitation solution to the viral cell lysate.
3. Vortex samples at high speed for 20 sec to mix the protein precipitation solution uniformly with the lysate.
4. Place sample into an ice bath for 5 min.
5. Centrifuge at 13,000–16,000 x g for 3 min. The precipitated proteins will form a tight pellet. If the protein pellet is not tight, repeat steps 3, 4, and 5.

DNA Precipitation

1. Leaving behind the precipitated protein pellet, pour the supernatant containing the DNA into a clean 1.5 ml microfuge tube that contains 300 µl 100% isopropanol (2-propanol). If DNA yield is expected to be low (<1 µg), add glycogen as a carrier to the isopropanol. We recommend adding 0.5 µl glycogen solution (20 mg/ml) per 300 µl isopropanol.
2. Cap the tubes and mix the sample by inverting gently 50 times. Incubate at room temperature at least 5 min.

3. Centrifuge at 13,000–16,000 x g for 5 min; depending on yield, the DNA may or may not be visible as a small, translucent pellet.
4. Pour off supernatant and drain tube on clean absorbent paper. Add 300 µl 70% ethanol, cap the tube, and invert it several times to wash the DNA pellet.
5. Centrifuge at 13,000–16,000 x g for 1 min. Carefully pour off the ethanol. Pellet may be loose; pour slowly and watch pellet to ensure that it stays in the tube.
6. Invert and drain the tube on clean absorbent paper and allow to air-dry 10–15 min.

DNA Hydration

1. Add 10 µl DNA hydration solution (10 µl will give a concentration of 100 ng/µl if the total yield is 1 µg DNA).
2. Rehydrate DNA by incubating sample for 1 hr at 65°C or overnight at room temperature. Tap tube periodically to aid in dispersing the DNA.
3. Before use, vortex sample vigorously for 5 sec and pulse-spin. Pipet sample up and down several times to ensure adequate mixing.
4. Store purified DNA sample at 4°C. For long-term storage, store at –70 to –80°C.

Ordering Information

Catalog #	Description
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

Related Products

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
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
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