

Isolation of Genomic DNA from Cultured-Cell Supernatant Containing Virus (1 ml Viral Sample)

AquaPure® Genomic DNA Isolation Kit

Catalog # 732-6340

Expected yield: 2–50 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 1 ml residual liquid to thoroughly resuspend the pellet. Keep sample on ice or store frozen at –70 to –80°C.

Cell Lysis

1. Add 5 ml cell lysis solution to a sterile 15 ml centrifuge tube containing 1 ml virus.
2. Incubate at 65°C for 15 min to complete lysis. Or, if maximum yield is required, add 30 µl 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 30 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 2 ml 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 5–15 min.
5. Centrifuge at 2,000 x g for 10 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 15 ml centrifuge tube that contains 6 ml 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 10 µl glycogen solution (20 mg/ml) per 6 ml 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 2,000 x g for 10 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 6 ml 70% ethanol and invert the capped tube 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 100 µl DNA hydration solution (100 µl will give a concentration of 250 µg/ml if the total yield is 25 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 and then transfer to a 1.5 ml microfuge tube.
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