

DNA Isolation from 300–450 mg Dried or 600–1,200 mg Fresh Leaf Tissue

AquaPure® Genomic DNA Isolation Kit

Catalog #732-6340

Expected yield range: 90–900 µg DNA

Method

Cell Lysis

1. Add 300–450 mg dried tissue (finely ground) or 600–1,200 mg frozen tissue (which may be finely ground with a mortar and pestle in liquid nitrogen), or 600–1,200 mg fresh tissue to a 50 ml tube. Work quickly and keep tissue cold to minimize DNase activity. Note: it may be necessary to vary the amount of starting material depending upon the species, age, tissue preparation, and genome size.
2. Add 18 ml cell lysis solution to the leaf tissue. For dried tissue, cap the tubes and vortex 1–3 sec to wet the tissue. For unground tissue, homogenize using 30–50 strokes with a tube pestle.
3. Incubate cell lysate at 65°C for 60 min. After 30 and 60 min, invert capped tube 10 times.

RNase Treatment (Optional)

1. Add 90 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 min.

Protein Precipitation

1. Cool sample to room temperature.
2. Add 6 ml protein precipitation solution to the lysate.
3. Cap the samples and vortex vigorously at high speed for 20 sec to mix the protein precipitation solution uniformly with the cell lysate. Alternatively, invert a rack containing the samples 150 times (approximately 2 min) to mix the protein precipitation solution uniformly with the cell lysate. For species with high polysaccharide content, it may be necessary to incubate sample on ice for 5–15 min.
4. Centrifuge at 3,000 x g for 10 min. The precipitated proteins should form a tight, green pellet. The supernatant may range in appearance from a transparent brown to green, depending on the sample. If the pellet is not tight, repeat step 3, incubate on ice for 5 min, then repeat step 4.

DNA Precipitation

1. Leaving behind the precipitated protein pellet, pour the supernatant containing the DNA into a 50 ml centrifuge tube that contains 18 ml 100% isopropanol (2-propanol).

2. Mix the sample by inverting the capped tube gently 50 times.
3. Centrifuge at 3,000 x g for 5 min. The DNA will be visible as a pellet that ranges in color from off-white to dark green, depending on particulates that cosediment with the DNA.
4. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 18 ml 70% ethanol and invert the capped tube several times to wash the DNA pellet.
5. Centrifuge at 3,000 x g for 5 min. Carefully pour off the ethanol. Pellet may be loose, so pour slowly and watch the pellet to ensure that it stays in the tube.
6. Invert and drain the tube on clean absorbent paper and allow to air-dry 15 min.

DNA Hydration

1. Add 1 ml DNA hydration solution.
2. Rehydrate DNA by incubating sample 1 hr at 65°C, or alternatively, overnight at room temperature. Tap tube periodically to aid in dispersing the DNA. Transfer DNA sample to a clean 1.5 ml tube for storage.
3. If particulates are present in the rehydrated DNA sample, centrifuge at 13,000–16,000 x g for 5–10 min and transfer supernatant into a clean 1.5 ml tube. Store DNA at 2–8°C.

Ordering Information

Catalog #	Description
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 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