DNA Isolation from 5–20 mg of Paraffin-Embedded Tissue

AquaPure® Genomic DNA Tissue Kit

Catalog #732-6343

Method

Sample De-paraffinization

1. Place 5–10 mg (0.005–0.010 g) of finely minced tissue in a 1.5 ml capped tube. Add 300 µl xylene and incubate 5 min with constant mixing at room temperature.
2. Centrifuge at 13,000–16,000 x g for 1–3 min to pellet the tissue. Discard the xylene.
3. Repeat steps 1 and 2 twice (for a total of 3 xylene washes).
4. Add 300 µl of 100% ethanol to the tube and incubate 5 min with constant mixing at room temperature.
5. Centrifuge at 13,000-16,000 x g for 1-3 min to pellet the tissue. Discard the ethanol.
6. Repeat steps 4 and 5 (for a total of 2 ethanol washes).

Cell Lysis

1. Add 300 µl cell lysis solution, and homogenize using 30–50 strokes with a microfuge-tube pestle.
2. Incubate lysate at 65°C for 15–60 min.
3. If maximum yield is required, 1.5 µl proteinase K solution (20 mg/ml) may be added to the lysate. Mix by inverting capped tube 25 times and incubate at 55°C until tissue particulates have dissolved (3 hr to overnight). If possible, invert tube periodically during the incubation.

RNase Treatment

1. Add 1.5 µl RNase A solution (4 mg/ml) 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 RNase A-treated cell lysate.
3. Vortex capped tube vigorously at high speed for 20 sec to mix the protein precipitation solution uniformly with the cell lysate.
4. 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 visible, repeat step 2 followed by incubation on ice for 5 min, then repeat step 3.

DNA Precipitation

1. Leaving behind the precipitated protein pellet, pour the supernatant that contains the DNA into a clean 1.5 ml microfuge tube containing 300 µl 100% isopropanol (2-propanol). If DNA yield is expected to be low (<1 µg), add 0.5 µl glycogen solution (20 mg/ml) to the isopropanol.
2. Cap the tube and mix by inverting gently 50 times.
3. Centrifuge at 13,000–16,000 x g for 5 min.
4. Pour off supernatant and drain tube on clean absorbent paper. Add 300 µl 70% ethanol and invert 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, 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 for 15 min.

DNA Hydration

1. Add 20 µl DNA hydration solution (20 µl will give a concentration of 100 ng/µl if the total yield is 2 µg DNA).
2. Allow DNA to rehydrate overnight at room temperature. Alternatively, heat at 65°C for 1 hr. Tap tube periodically to aid in dispersing the DNA.
3. Store DNA at 2–8°C.

Ordering Information

Catalog #	Description
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
Related Products	
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
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

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