

## Genomic DNA Isolation Protocol for 40 µl Avian Whole Blood

### AquaPure® Genomic DNA Isolation Kit

Catalog #732-6340

Expected yield range: 100–300 µg DNA

#### Method

##### Cell Lysis

1. Use fresh blood within 10 sec of venipuncture to avoid clotting. Alternatively, collect whole blood in EDTA to prevent the blood from clotting and reduce DNA degradation. Blood may be either fresh or frozen. Store fresh blood sample at 2–8°C for not more than 5 days to obtain optimal DNA isolation results.
2. Add 40 µl whole blood to a 15 ml microfuge tube containing 6 ml cell lysis solution. Quickly pipet up and down 3–5 times to lyse the cells. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C until the solution is homogeneous. Samples are stable in cell lysis solution for at least 18 months at room temperature.

##### RNase Treatment (Optional)

1. Add 30 µl RNase A solution to the cell lysate.
2. Mix the sample by capping the tube and inverting it 25 times, then 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 RNase A-treated cell lysate.
3. Vortex vigorously at high speed for 20 sec to mix the protein precipitation solution uniformly with the cell lysate.
4. Centrifuge at 2,000 x g for 10 min. The precipitated proteins will form a tight pellet. If the protein pellet is not visible or 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 15 ml centrifuge tube containing 6 ml 100% isopropanol (2-propanol).
2. Mix the sample by gently inverting the capped tube 50 times.

3. Centrifuge at 2,000 x g for 3 min; the DNA will be visible as a small white pellet.
4. Pour off the 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 2,000 x g for 1 min. Carefully pour off the ethanol. Pellet may be loose, so pour slowly and watch to ensure that pellet 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 500 µl DNA hydration solution (500 µl will give a concentration of 400 µg/ml if the total yield is 200 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
732-6340	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

##### 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 <sup>6</sup> 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 <sup>6</sup> 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