

PUREGENE® DNA Purification Kit

DNA Purification Protocol From 10 ml Buffy Coat Prepared from 10 ml Whole Blood

50 ml Tube Prep - Expected Yield Range 200-400 µg DNA

Cell Lysis

Thaw buffy coat for 1-2 minutes at 37°C .

If buffy coat preparation contains red blood cells, treat sample with **RBC Lysis Solution** by beginning with Step 1 below. If buffy coat preparation is clean and free of red blood cells, pipet 500-850 µl sample into 50 ml tube containing 10 ml Cell Lysis Solution as instructed in Step 4 below.

1. Add buffy coat preparation (500-850 µl) obtained from a 10 ml whole blood sample to a 50 ml tube containing 3 parts **RBC Lysis Solution** . Invert to mix and incubate 5 minutes at room temperature; invert again at least once during the incubation.
2. Centrifuge for 10 minutes at 2,000 x g. Remove supernatant leaving behind the visible white cell pellet and about 200-400 µl of the residual liquid.
3. Vortex the tube vigorously to resuspend the cells in the residual liquid; this greatly facilitates cell lysis in Step 4 below.
4. Add 10 ml Cell Lysis Solution to the resuspended cells and pipet up and down to lyse the cells. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C or room temperature until the solution is homogeneous. Samples are stable in **Cell Lysis Solution** for at least 2 years at room temperature. Let stand at least 1-2 minutes.

Protein Precipitation

1. Cool sample to room temperature.
2. Add 3.33 ml **Protein Precipitation Solution** to the cell lysate.
3. Vortex vigorously at high speed for **20 seconds** to mix the **Protein Precipitation Solution** uniformly with the cell lysate.
4. Centrifuge at 2,000 x g for 5 minutes. The precipitated proteins will form a tight dark brown pellet. If the protein pellet is not tight, repeat Step 3 followed by incubation on ice for 5 minutes and then repeat Step 4.

DNA Precipitation

1. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 50 ml tube containing 10 ml **100% Isopropanol** (2-propanol).
2. Mix the sample by inverting gently 50 times until the white threads of DNA form a visible clump.
3. Add 1ml **70% Ethanol** to a 1.5ml centrifuge tube. Using a Pasteur pipet transfer the strands/clump of DNA from the 50ml conical to the centrifuge tube.
4. Pipet off ethanol. Tilt centrifuge tubes and allow to dry for 15 minutes.

DNA Hydration

1. Add 500 µl **DNA Hydration Solution**.
2. Rehydrate DNA by incubating at 65°C for 2 hours and overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
3. For storage, sample may be centrifuged briefly and then transferred to a 1.5 ml tube. Store DNA at 4°C. For long term storage, store at -20°C or -80°C.