

PUREGENE[®] DNA Isolation Kit

DNA Isolation Protocol For 300 µl Whole Blood

Microfuge Tube Prep - Expected Yield Range 5-15 µg DNA

Cell Lysis

1. Add 300 µl whole blood (or bone marrow) to a 1.5 ml microfuge tube containing 900 µl **RBC Lysis Solution**. Invert to mix and incubate 10 minutes at room temperature; invert again at least once during the incubation.
2. Centrifuge for 20 seconds at 13,000-16,000 x g. Remove supernatant with a pipette leaving behind the visible white cell pellet and about 10-20 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 300 µl **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 18 months at room temperature.

RNase Treatment (Optional)

1. Add 1.5 µl **RNase A Solution** to the cell lysate.
2. Mix the sample by inverting the tube 25 times and incubate at 37°C for 15 minutes.

Protein Precipitation

1. Cool the sample to room temperature.
2. Add 100 µl **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 13,000-16,000 x g for 3 minutes. The precipitated proteins should 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 1.5 ml tube containing 300 µl 100% **Isopropanol** (2-propanol).
2. Mix the sample by inverting gently 50 times.
3. Centrifuge at 13,000-16,000 x g for 1 minute; the DNA will be visible as a small white pellet.
4. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 300 µl 70% **Ethanol** and invert the tube several times to wash the DNA pellet.
5. Centrifuge at 13,000-16,000 x g for 1 minute. Carefully pour off the ethanol. *Pellet may be loose so pour slowly and watch pellet.*
6. Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.

DNA Hydration

1. Add 100 µl **DNA Hydration Solution** (100 µl will give a concentration of 100 µg/ml if the total yield is 10 µg DNA).
2. Allow DNA to rehydrate by incubating at 65°C for 1 hour and/or overnight at room temperature. Tap tube periodically to aid in dispersing the DNA.
3. Store DNA at 2-8°C.



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