

DNA EXTRACTION USING GUANIDINE THIOCYANATE

SOLUTIONS

CELL LYSIS BUFFER

100 mM NaCl

100 mM Tris-Cl pH 8.0

25 mM EDTA pH 8.0

0.5% SDS

Autoclave the NaCl, Tris and EDTA before making the buffer. Filter sterilize.

PROTEIN PRECIPITATION SOLUTION

4M Guanidine Thiocyanate

0.1 M Tris-Cl, pH 7.5

Heat the solution to get the guanidine thiocyanate into solution. Filter sterilize, but DON'T USE cellulose acetate filters. Wes says to use cellulose nitrate. Polyethersulfone works okay too. The cellulose acetate filters dissolve.

PROTEINASE K (20 mg/mL)

RNASE A (4 mg/ml)

ISOPROPANOL

ETHANOL (70%)

1X TE (10mM Tris-Cl, pH 8.4, 1 mM EDTA) or 10 mM Tris-Cl (pH 8.4)

METHOD

Cell Lysis and RNase Treatment

1. Place 10 mg of tissue in a 1.5 mL microcentrifuge tube containing 300 μ L of CELL LYSIS SOLUTION (see recipe above). Macerate the tissue as much as possible.
2. Add 1.5 μ L PROTEINASE K (20 mg/mL). Mix with a brief vortex. Incubate 3-6 hours (or overnight if you really need to) at 55°C.
3. Homogenize by vortexing gently.
4. Add 1.5 μ L RNASE A (4mg/mL). Incubate at 37°C for 30-45 minutes.
5. Cool the sample to room temperature.

Protein Precipitation

6. Add 100 μ L PROTEIN PRECIPITATION SOLUTION to the cell lysate mixture. Vortex vigorously to mix the tube contents (10-20 seconds).
7. Centrifuge at the highest speed (13000 rpm) for 5 minutes. Repeat if the protein pellet is not tight.
8. Pour off or aspirate the supernatant (which contains the DNA) into a new 1.5 mL microcentrifuge tube.

DNA Precipitation

9. Add 300 μ L 100% isopropanol. Mix by inverting gently 50 times.
10. Centrifuge at 13000 rpm for 5 minutes.
11. Pour off the supernatant, careful to leave the pellet behind.
12. Add 300 μ L 70% ethanol and invert the tube several times to wash the pellet.
13. Centrifuge at 13000 rpm for 5 minutes. Pour off the supernatant.
14. Air-dry for several hours (or overnight if you really need to).

DNA Hydration and storage

15. Add 50-200 μ L of 1x TE or 10 mM Tris-Cl, pH 8.0. Incubate overnight at room temperature or one hour at 65°C.
16. Store long-term at -20°C or -80°C, otherwise store at 4°C.

Notes:

This protocol gives results identical to those of the Puregene kit when comparing extracted DNA using the two techniques on agarose.