DNAzol® DNA Extraction Technique

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READ COSHH FORM AND RISK ASSESSMENT BEFORE STARTING THIS PROCEDURE

The DNAzol reagent contains the chaotropic agent guanidine thiocyanate and a detergent mixture in a lysing solution, which permit selective precipitation of DNA from a cell lysate.

Method:

1. Remove the samples from the freezer and allow to thaw. Once thawed, replace in the freezer for 5-10 minutes to refreeze. Repeat twice more.

2. Add 100µl of DNAzol reagent to each sample tube. Grind the samples using either a sterile pipette tip or a clean plastic pestle, changing the pestle between samples.

3. Add another 150µl of DNAzol reagent to the sample tube and mix twice by inversion.

4. Centrifuge the samples at 13,000rpm for 10 minutes. Transfer the supernatant to new, labelled tubes, taking care not to disturb the protein pellet by pointing the pipette tip towards the opposite side of the tube. Repeat to remove the last of the tissue debris.

5. Precipitate the DNA by adding 125µl of cold 100% ethanol to each sample. Invert the tube gently ten times to mix.

6. Leave the samples at room temperature for 5 minutes.

7. Centrifuge the samples at 13,000rpm for 10 minutes to pellet the DNA. Discard the supernatant, taking care not to disturb the pellet.

8. Wash the DNA pellet once with 250µl of 95% ethanol. Mix gently by inversion and centrifuge at 13,000rpm for 5 minutes. Discard the supernatant, taking care not to disturb the pellet as before.

9. Dry the pellet in a 60˚C oven for approximately 5 minutes (leave the tube caps open and cover the tubes loosely with foil).

10. Redissolve the DNA pellet in 50µl of 1xTE. Mix by tapping gently and allow to stand for at least 10 minutes before storing at 4˚C or -20˚C.