

Removing DNA from DNA/RNA mixtures:

Reagents:

10 X digestion buffer (20 mM MgCl₂, 10 mM ZnCl₂, 500 mM Tris, pH 7.2)

DNase I

Protein precipitation solution (10 M ammonium acetate)

Ice-cold Isopropanol

Ice-cold Ethanol (70 %)

DEPC treated water or 1 x TE buffer

1. Add 1/10 volume of 10 X digestion buffer and 1 U of DNase I, mix by flicking tubes and spin briefly (do not vortex this will reduce the activity of DNase).
2. Incubate at 37°C for 30 min.
3. To remove enzyme add 1 volume of protein precipitation solution, vortex for 10s on low speed and place in ice-bath for 5 min.
4. Centrifuge at top speed (16,100 x g) at 4°C for 10 min and transfer supernatant to a new tube.
5. Add 1 volume of ice-cold isopropanol and invert tube about 30 times to mix.
6. Centrifuge at top speed (16,100 x g) at 4°C for 10 min and remove supernatant.
7. Rinse pellet twice with 70 % ice-cold ethanol spinning between rinses.
8. Dry pellet slightly in vacuum dessicator (about 2 min).
9. Resuspend pellet either in sterile H₂O or in 1 X TE buffer.
10. Store at -20°C short term or -80°C long term.