LYSATE: NUCLEASE TREATMENT AND DESALTING

Stock Solutions:

- 0.1 M CaCl2
- 15,000 U/ml staph nuclease
- 0.1 M EGTA

Other Odds and Ends

- 5 ml plastic syringe
- nitex cloth
- Eppendorf tubes
- Corning 50 ml sterile tubes
- Sephadex G-25 (fine) - Pharmacia
- untreated lysate
- iquid nitrogen and appropriate flask

Preliminary Preparation:

Cut out a small square of nitex to fit onto the end of syringe barrel. Warm up end of barrel near (but not in) flame of bunsen burner. Place on nitex square and make sure it is firmly affixed.

Cut out a hole from cap of Corning tube so that barrel of syringe may rest in Corning tube.

Add autoclaved water to Sephadex (>2 X volume of beads) and let sit at least 20 min.

Protocol for 1 ml lysate:

1. Add Sephadex to syringe barrel to slightly above 5 ml mark. Put syringe into Corning tube. Spin in low speed centrifuge at 4øC at 1000 rpm for 1 min. If beads are compacted below 5 ml mark, add more of swollen beads and repeat. Keep cold.
2. Add 10 ul stock CaCl2 (eff. conc. 1 mM).
3. Add 10 ul nuclease to lysate and let sit at room temperature for 10 min.
4. Add 20 ul stock EGTA (eff. conc. 2 mM).
5. Put 2 ul of stock EGTA into bottom of Corning tube (eff. conc. 0.2 mM).
6. Take up lysate into a P-1000 and dispense into centre of bed, avoiding sides of barrel. Spin in low speed centrifuge at 1000 rpm for 1 min. at 4øC.
7. Collect lysate from bottom of Corning tube and aliquot into Eppendorf tubes. Quick freeze in liquid nitrogen.