**ENZYMES**

Lysozyme

*Stock solution.* 50 mg/ml in water. Dispense into aliquots and store at −20°C. Discard each aliquot after use; do not refreeze.

RNase That Is Free of DNase

Dissolve pancreatic RNase (RNase A) at a concentration of 10 mg/ml in 10 mM Tris·Cl (pH 7.5) and 15 mM NaCl. Heat to 100°C for 15 minutes and allow to cool slowly to room temperature. Dispense into aliquots and store at −20°C.

DNase That Is Free of RNase

Unfortunately, many commercial preparations of pancreatic DNase I, even those sold as "RNase-free," are contaminated by amounts of ribonuclease that are sufficient to cause significant degradation of high-molecular-weight RNA. Two methods, given below, are available to remove the contaminating RNase activity.

*Affinity chromatography on agarose-coupled 5'- (4-aminophenyl-phosphoryl) uridine 2'(3') phosphate (Maxwell et al. 1977).*

1. Equilibrate 10 ml of agarose-5'- (4-aminophenyl-phosphoryl) uridine 2'(3') phosphate (commercially available from Miles-Yeda Laboratories) with 0.02 M sodium acetate (pH 5.2). Make a column in a 25-ml disposable syringe.

2. Dissolve 20 mg of pancreatic DNase I (DPFF, Worthington Biochemicals) in 1 ml of 0.02 M sodium acetate (pH 5.2).

3. Apply the solution of DNase I to the column and elute with 0.02 M sodium acetate (pH 5.2) at room temperature. Collect 1-ml fractions into RNase-free tubes (see pages 190, 437) until all material absorbing at 280 nm has eluted from the column.

4. Pool the fractions that contain protein. Read the OD

450 and calculate the concentration of protein (1 OD

450 = 1 mg of protein). Dispense the enzyme preparation into small aliquots and store at −20°C.
**Adsorption to macaloid.** Macaloid, a clay that has been known for many years to adsorb RNase, is available from the National Lead Company, Houston, Texas. It is prepared as follows (Schaffner 1982):

a. Suspend 0.5 g of macaloid powder in 50 ml of sterile 50 mM Tris·Cl (pH 7.6). Heat to 100°C for 5 minutes with constant agitation.

b. Centrifuge at room temperature for 5 minutes at 2500g.

c. Discard the supernatant. Resuspend the sticky pellet completely in 40 ml of sterile 50 mM Tris·Cl (pH 7.6).

d. Repeat the centrifugation and washing steps twice more.

e. Centrifuge the suspension for 15 minutes at 3500g.

f. Resuspend the pellet in 30 ml of sterile 50 mM Tris·Cl (pH 7.6). The final concentration of macaloid is 16 mg/ml. The suspension may be stored indefinitely at 4°C.

The macaloid suspension is used in the following steps to remove contaminating RNase activity from DNase.

1. Dissolve 100 mg of DNase I (DPFF, Worthington Biochemicals) in 5 ml of:

   - 20 mM Tris·Cl (pH 7.6)
   - 50 mM NaCl
   - 1 mM dithiothreitol
   - 100 μg/ml BSA
   - 50% glycerol

2. Add 15 ml of ice-cold 50 mM Tris·Cl (pH 7.6). Mix gently.

3. Add 7.0 ml of an ice-cold, well-dispersed suspension of macaloid and mix on a rotating wheel for 30 minutes at 4°C.

4. Centrifuge for 10 minutes at 8000g at 0°C. Decant the supernatant into a fresh tube.

5. Add another 7.0 ml of macaloid suspension and mix as before.

6. Centrifuge for 15 minutes at 12,000g.

7. Carefully remove the supernatant and mix it gently with an equal volume of ice-cold, sterile glycerol.

8. Dispense into small aliquots and store at −20°C. The concentration of DNase I is approximately 3.0 mg/ml.