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 chromotography 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_{280} and calculate the concentration of protein (1 $OD_{280} \simeq 1$ 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 2500*g*.

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 3500*g*.

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 8000*g* 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 12,000*g*.

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.