

NUCLEASE TREATMENT OF RABBIT RETICULOCYTE LYSATE

Materials:

Keep the following solutions cool in an ice bath:

50 mM CaCl₂

100 mM K - EGTA pH 7.0

Hemin solution - made according to CSH Cloning Handbook, p. 346.

Keep the following solutions frozen at -80°C until just before use:

Rabbit Reticulocyte Lysate from Promega

Micrococcal Nuclease, 15,000 U/ml in H₂O

Creatine Phosphokinase 10 mg/ml (1250 U/ml) in 50% glycerol

Dry ice/Ethanol bath

Ice bath

20°C bath

1. Thaw an aliquot of micrococcal nuclease, put on ice.
2. Rapidly thaw an aliquot of reticulocyte lysate and put on ice.
3. Per one ml of lysate, add:
 - 10 µl Hemin solution
 - 20 µl CaCl₂
4. Mix, then add per one ml lysate: 6 µl micrococcal nuclease
5. Mix, then incubate at 20°C for 15-20 min. then put on ice.
6. Add 20 µl EGTA per ml lysate.
7. Add 30 µl of creatine phosphokinase per ml of lysate (Thaw the CPK immediately before use, and discard the unused portions after thawing).
8. Mix gently, then aliquot into pre-chilled Eppendorf tubes or NUNC vials, in 100 - 200 µl aliquots.
9. Snap freeze the aliquots in dry ice/ethanol bath. Store the aliquots at -80°C or under liquid N₂ (only NUNC vials can be used in liquid N₂).

It is important to work rapidly, especially after the CPK has been thawed.

Lysate is stable at -80°C for at least one year, and stable indefinitely under liquid N₂.

(c.f. Harvey Lodish Cookbook; Cold Spring Harbor Cloning Handbook; and Pelham, H.R.B. and Jackson, J.R., Eur. J. Biochem. (1976) 67: 247-256.)