PELLETED POLYSOMES

A common method for analysing mRNA is to translate the population of message isolated from the cell of interest in a cell-free translation system. It is sometimes useful to use isolated polysomes as a source of mRNA rather than purified RNA. In this case it is necessary to use a translation extract which is free of ribosomes (S_{150} fraction). Re-initiation does not often occur in this system.

The following protocol yields both an S_{150} fraction and a pelleted polysome fraction. Also included is a protocol form Dr. Masayuki Yamamoto (Engel lab 1984-1985) for preparation of pelleted polysomes from anemic hens. You may want to titrate the amount of polysomes needed in a translation. Dr. Yamamoto suggests 0.5 A_{260} units.

Protocol #1

1. Wash blood cells extensively in: 140mM NaCl
7.5 mM KCl
5.2 mM $MgCl_2$
Spin in between at 8.000g for 10 min in corex tubes at 4°C.

2. Lyse cell pellet in 4 cell volume of: 2 mM $MgCl_2$
0.1 mM EDTA pH 7.0
1mM DTT
Swirl gently.

3. Pellet cellular debris at 16,000 g for 20 min. at 4°C.
   A. The supernatant is total extract this can be frozen in 100µl aliquots at -70°C to be used for in vitro translations.
   B. To prepare S_{150} fraction and pelleted polysomes:

1. Supernatant (total extract) is cleared by ribosomes by a high spin 143,000g/3 hrs. which is accomplished using a SW50.1 rotor at 45,000 rpm at 4°C.
2. The pellet from this spin is the polysomes
   a. Resuspend these in; 0.25M sucrose
1 mM DTT
0.1 mM EDTA pH7
   b. Determine A_{260} and resuspend to 150-250 A_{260} units/ml
   c. Freeze at -70°C.
3. The supernatant is recleared under the same conditions. Use top 75% for S_{150} extract. Store in 100µl aliquots at -70°C.

Protocol #2 Yamamoto Method (personal communciation)

Preparation - All solutions must be kept COLD

Isotonic buffer (sterile) resuspension buffer (sterile)

1x polysome buffer
15% sucrose
2mM DTT - Add fresh
4 mg/ml heparin - Add fresh

1x polysome buffer
0.2M sucrose

<u>50% sucrose/polysome buffer</u>

<u>Polysome buffer</u> (sterile)
85 mM KCl
5 mM $MgCl_2$
10 mM Hepes KPH pH 7.4

<u>RSB</u>
10 mM KCl
1.5 mM $MgCl_2$
10 mM Hepes KOH pH 7.4

Cold Ti60.1 + <u>tubes</u>

1. Wash blood extensively with: 140mM NaCl
   7.5 mM KCl
   5.2 mM $MgCl_2$
   Spin 8.000g for 10 min. in Corex tubes at 4°C.
2. Resuspend in 2 vols RSB.
   Mix gently on ice.
3. Add 1 vol. isotonic buffer.
4. Spin in SA600 rotor at 10K rpm/10 min/4°C ~ SAVE SUPERNATANT.
5. Place up to 30 mls in cold Ti60 sealable tube.
6. Underlay w/10mls 50% sucrose in polysome buffer.
7. Spin at 50K rpm for 4 hours at 4°C..
8. Pierce top of tube with needle
   syringe, extract a few mls of supernatant.
   Cut tube and pour off material.
9. Pellet should be whitish.
10. Resuspend in 200 µl of polysome buffer containing 0.2 M sucrose .
11. Spin in microfuge to remove "garbage".