YCCEH Protocols

POLYSOME PREPARATION FROM EUKARYOTIC CELLS

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Reagents

TMK-100 (Tris/Mg^{2+}/K^{+}) lysis buffer

- 10 mM Tris×Cl, pH 7.4
- 5 mM $MgCl_2$
- 100 mM KCl
- 1% (v/v) Triton X-100
- 0.5% (w/v) deoxycholate
- Prepare in nuclease free (DEPC or molecular grade) water.
- Right before use, add:
- 1 U/ml ribonuclease inhibitor (like RNAsin from Promega)
- 2 mM dithiothreitol

Sucrose gradient solutions, 10% and 60% (w/v)

- 10% or 60% (w/v) sucrose
- 100 mM KCl
- 5 mM $MgCl_2$
- 2 mM dithiothreitol
- 20 mM HEPES×KOH, pH 7.4
- Prepare in nuclease free water (DEPC or molecular grade)

Overview

This protocol described preparing polysome fractions from cell lysates to study translation. Cells are lysed in a lysis buffer with detergent and cleared of cellular debris on tabletop centrifuge. The lysate is then fractionated by ultracentrifugation and collected with continuous monitoring of absorbance at 254 nM.

Procedure

We typically use 40-50 million cells per prep. This will be approximately 15 cm dishes x 4 for adherent cell types like HeLa or 293T or about two T75 flasks (35 ml media each) for suspension cells like K562. It is important to grow these cells in log phase right up to the lysis stage. Changing media the day prior to harvest (about 16 hours earlier) is recommended.

10 minutes prior to harvest add cycloheximide at final concentration of 100 ug/ml to the cells and return to 37-degree incubator.

Harvest cells rapidly at the end of 10 minutes. Keep cells at 4 degrees from this point onwards. We prefer to collect cells and lyse after collection but direct lysis of adherent cells on plate is also possible. For adherent cells, use trypsin or EDTA as is standard protocol and wash once with PBS/ cycloheximide (PBS with cycloheximide at 100 ug/ml final concentration). For suspension cells, spin down cells in 50 ml conical tubes and wash once with PBS/ cycloheximide.

After wash, lyse cells with about 900 ul of TMK100 buffer (see recipe below). For end applications that require RNA isolation, add RNAse inhibitors as suggested, this may not be necessary if you were to use the fractions for protein analysis. Keep lysis buffer and lysates at 4 degree Celsius or ice at all times.

Prepare sucrose gradient on the gradient master with 10 % and 60 % sucrose solutions. This takes several hours and hence needs to be done prior to starting cell lysis

Clear the cell lysates just prior to loading on the gradient by spinning at max speed (~ 13000 rpm) on standard tabletop micro centrifuge at 4 degree Celsius.

Load the cleared lysate to the gradient and proceed to ultra- centrifugation (222,228 x g or 36,000 rpm on SW41Ti rotor), for 2 hours at 4 °C using.
Note: In order not to disrupt sucrose gradients, “low” brake option should be selected.

Proceed to fractionation with fraction collector with continuous monitoring of absorbance at 254 nm (We typically collect 24 fractions).

Protocol adapted from:
Analysis of eukaryotic translation in purified and semi purified systems. Current Protocols in Cell Biology 2001 May;Chapter 11:Unit 11.9.
http://www.ncbi.nlm.nih.gov/pubmed/18228314