IN-VITRO TRANSLATION

Prepare a master mix for n + 1 number of translations:

n = 1
4.0 µl sterile distilled water
1.0 µl 25X K-Mg
2.0 µl translation cocktail
3.0 µl ^{35}S-met (5 mCi/ml)
10.0 µl nucleased rabbit reticulocyte lysate

20.0 µl total per translation

Translation cocktail:

6.25 mM Spermidine HCl
100 mM Creatine phosphate
0.3125 mM amino acids (methionine minus)
25 mM DTT
250mMHEPES(pH7.4)

Add 20 µl master mix to 5 µl of a sample of (water + RNA). Typically, 0.5-1.0 µg of polyA+ RNA or 5-20 µg of total RNA is used for each 25 µl translation. 5 µl of water is used for the "endogenous" translation in the absence of RNA.

Incubate at 30°C for 90 minutes.

Note:

1. 25X K-Mg is a solution of potassium chloride and magnesium chloride (acetate salts can be used as well) such that 1X is the optimal concentration of K and Mg for the lysate. Typically K optimum is between 60-100 mM and Mg optimum can be from 0-0.1 mM.

2. Avoid repeated freeze/thaw of the methionine by distributing the stock into small aliquots. The chemical stability can be improved by diluting the stock to 5 mCi/ml with 1mM dithiothreitol. The amount of label can be increased to 35uCi or more per translation if so desired.

In order to determine the level of incorporation of ^{35}S-met.:

1. Add a sample (2-5 µl) of the translation to 0.5 ml of water.

2. Add 0.5 ml of 1.0 N NaOH (to hydrolyze amino-acyl tRNA) and 80 µl of 30% hydrogen peroxide (to decolorize).

3. Incubate at 37°C for 20 min.

4. Add 2.0 ml of 25% trichloroacetic acid containing carrier amino acids (2 mg/ml methionine or 3% casamino acids).

5. Chill on ice at least 20 min.

6. Collect the precipitate on glass fibre or nitrocellulose filters. Wash the filters with 10% TCA followed by 95% ethanol.

7. Allow the filters to dry. Count by liquid scintillation using an organic counting cocktail.

8. Typically, RNA should increase the incorporation of label 20-80 fold over endogenous. The conditions used here give 500-2000 cpm for endogenous translation and 30,000-100,000 cpm for samples with RNA in 5µl.

9. For analysis by SDS-PAGE, dilute up to 5 µl of the translation assay with 20-30 µl of sample buffer. Because of the large amount of protein in the lysate it is easy to exhaust the capacity of the SDS in the sample buffer; I therefore recommend using sample buffer that contains 4% SDS.