# *Translational extracts active biologically in vitro obtained from eukaryotic monolayers cells: a versatile method for viral RNA studies*

Journal of Virological Methods 92: 177-181 (2001)

Favre, D. & Trépo, C.

1) Washing buffer and buffer for permeabilization (with lysolecithin).

| Compound | Stock | For 100 ml | |
|---|---|---|---|
| 20 mM Hepes pH 7.4 | 1 M | 2 | ml |
| 150 mM sucrose | 1 M | 15 | ml |
| 33 mM $NH_4Cl$ | 1 M | 3.3 | ml |
| 7 mM KCl | 250 mM | 2.8 | ml |
| water | | to 100 ml | |

2) Extraction buffer

| | | | |
|---|---|---|---|
| 100 mM Hepes pH 7.4 | 1 M | 100 | µl |
| 120 mM KOAc pH 7.4 | 5 M | 24 | µl |
| 2.5 mM MgOAc | 100 mM | 25 | µl |
| 1 mM DTT | 200 mM | 5 | µl |
| 2.5 mM ATP | 100 mM | 25 | µl |
| 1 mM GTP | 100 mM | 10 | µl |
| 100 µM S-ad-methionine | 10 mM | 10 | µl |
| 1 mM spermidine | 100 mM | 10 | µl |
| 10 mM creatine phosphate | 1 M | 10 | µl |
| 40 µM amino acids -met | 1 mM | 40 | µl |
| water | | to 1 ml | |

If translational extracts will be frozen and subsequently thawed, add to the above and to a final volume of 1 ml (add water accordingly):

| | | | |
|---|---|---|---|
| 100 mM sucrose | 100 mM | 100 | µl |
| CK | 48 mg/ml | 2 | µl |

Additional notes:

i) *potassium acetate* (KOAc) has to be buffered with acetic acid to pH 7.4

ii) employ *L-alpha lysophosphatidylcholine, palmitoyl* (Sigma) at 100 micrograms per ml in washing buffer for around 60 to 90 seconds onto the cells. Remove ALL the washing buffer by upright draining prior to the addition of the extraction buffer.
A 100 X stock (10 mg/ml) is prepared in 50% chloroform, 50% methanol and stored at -20° C.

iii) *Creatine kinase.*

**The preparation of biologically active creatine kinase is of the upmost importance for it's use in the in vitro translation system.**

First of all, resuspend the lyophilized enzyme in water containing 40 mM dithiotreitol (DTT) and 50 mM Tris pH 8. This is allowing the regeneration of a biologically active dimer containing two reduced subunits (Park, Y.-D. et al. *Biochem. Cell Biol.* **79**: 479-487, 2001; Hurne, A. M. et al. *J. Biol. Chem.* 275(33): 25202-25206, 2000).

In this SDS-PAGE (Coomassie blue), we can see that the 42 kDa monomer of the creatine kinase is mostly in the biologically active, reduced form; only a very small amount is under the oxidized form at 37 kDa. The significance of the 30 kDa polypeptide is unknown (degradation product ?).

The enzyme should be stored (as a 48mg/ml stock in 50% glycerol) at -15 to -18 degrees Centigrade and NEVER below -18° C.

The lyophilized, original stock from Fluka is kept at -18° C.

iv) *Preincubation of the cells in cell culture medium lacking methionine prior to extract preparation.*

Before the preparation of the translation extract, the subconfluent cells growing in 10 cm-in-diameter Petri dishes (Costar) are washed with PBS and then incubated in cell culture medium lacking methionine and containing 0.1 to 0.2 % FCS for around 30 to 45 minutes. The cells are then placed on ice for 15 min before extract preparation.

Naturally this has to be done if it is intended to label the proteins with $^{35}$S-methionine (translation grade) during translation.
If the proteins will be labeled with $^{35}$S-methionine/$^{35}$S-cysteine, a preincubation medium lacking both methionine and cysteine has to be employed accordingly.

iii) *amino acids –met* and *amino acids –met/-cys* are from Promega.

iv) For translation in vitro in general, please read the article written by Clemens M.J. and entitled "Translation of eukaryotic messenger RNA in cell-free extracts", in: Transcription and translation control, a practical approach, IRL Press, 1984.

## Example.

Translation of the hepatitis B virus (HBV) and the duck hepatitis B virus (DHBV) polymerase mRNA in translational extracts obtained from BHK cells. Extracts have also been treated with micrococcal nuclease (stars) before the translation of the exogenous mRNAs.