

3 RABBIT RETICULOCYTE LYSATE CELL-FREE TRANSLATION

3.1 Introduction

Using rabbit reticulocyte lysates, any mRNA, either purified from tissue or synthesized in vitro from a cloned cDNA, can be expressed. It is basically a total lysate of reticulocytes with membranes centrifuged out and the supernate treated with hemin and staph micrococcal nuclease, the former to prevent formation of an inhibitor of translation and the latter to destroy endogenous RNA, freeing the extract to synthesize whatever RNA you wish to program the system with. This extract or lysate contains ample quantities of the components required for translation, e.g. ribosomes, initiation and elongation factors, etc. To this is added free amino acids, at least one of which is radioactive (typically methionine) to allow detection of newly synthesized proteins. Energy substrates and a regenerating system must also be added to drive protein synthesis. The ionic conditions are optimized for translation, your RNA or transcript is added and off you go.