3.3 Protocol for Translation in RRL

1. Prepare a detailed protocol of the experiment indicating title, date, purpose of experiment, list of tubes, list of components to add, volumes of each component to add to each, total volume, master mix components and volumes, post translational procedures (exactly what you intend to do with each tube after translation), order of samples to be loaded on gels. For a sample protocol see below.
2. Prepare mRNA or synthetic transcripts from SP6 polymerase reaction. Store frozen and handle on ice. See TRANSCRIPTION/TRANSLATION section 1.
3. Assemble all reagents on ice, and prepare a master mix as follows:

For 10 uL translation

CB 20X (SP6-linked, RRL)	0.5 uL
Energy Mix (5X)	2.0 uL
RRL (digested/desalted as above)	4.2 uL
tRNA calf liver 10 mg/mL	0.1 uL
Creatine kinase	0.1 uL
RNAsin 20 U/uL	0.1 uL
200X PIN (protease inhibitors)	0.1 uL
H2O	1.4 uL
TOTAL	8.5 uL

Now aliquot the master mix to all tubes on ice, then add the H2O, then the transcript (1 ul/10 ul reaction) and finally, the membranes 0.5 ul (or 0.5 eq.), if indicated. Vortex gently and incubate at 25øC for 1 hr. Transfer tubes to ice for aliquoting and post-translational procedures.