5.3 Immunoprecipitation of translation products

There are two general approaches to immunoprecipitation of translation products. The first is under nondenaturing conditions, the second involves TCA precipitation, solubilization and denaturation in SDS with excess SDS taken up in micelles of Triton before adding antiserum. The former is generally dirtier but doesn't necessarily require an antisera recognizing the denatured chain, while the latter is cleaner but requires an antibody against the denatured chain. An additional advantage of the latter approach is that it can be used subsequent to TCA precipitation of proteins which makes it a useful approach following post-translational proteolysis of samples where TCA can be used to kill the protease.

A. Triton solubilization/nondenaturing immunoprecipitation

1. To translation reactions are added 1/4 volume 5 X (or 10 X volume 1 X) concentrated immunoprecipitation buffer called TXSWB (Triton-Sepharose wash buffer, pronounced 'tick swib') supplemented with 1% Trasylol 90.1 mg/ml final conc or 1 mM PMSF (100 mM stock in EtOH).
2. Add the antibody (usually 1 ul of antiserum and usually to translation volumes of anywhere from 5 to 25 ul. If you are trying to immunoprecipitate a minor product of a total cellular RNA translation you may have to scale up significantly, e.g. up to 10 ul of antisera into 250 ul of translation reaction. In some circumstances it is important to pre-spin for 1 min in microfuge to clear a non-specific precipitate forms after several hours in the cold, and then transfer the supernate before adding antiserum. However, we find this only to be necessary for immunoprecipitations from oocyte homogenates and not from cell free translation products. Preincubate oocyte homogenates 4-12 hrs, spin, then add antibody to the supernate.
3. After addition of antiserum. incubate for at least 4 hrs and up to 12 hrs at 4øC.
4. Add protein A Affigel (5 ul of a 50% slurry freshly washed in 1 X TXSWB for tubes containing 3 ul or less of antiserum, add more in the ratio of 5 ul 50% slurry per additional 2 ul antiserum) and incubate at 4øC for 2 hr on rotator. For dispensing the protein A Sepharose, be sure and cut off the tip of the yellow pipette person tip so that the beads can enter without hindrance.
5. Carry out three sequential washes of the Affigel with 1 ml each of 1 X TXSWB spinning down for 5 seconds each time in the microfuge then aspirating the sup with a pulled out Pasteur pipette under suction. Leave about 50 ul in the bottom of the tube each time.
6. Wash the pellet twice with 1 ml 0.1 M NaCl 0.1 M Tris pH 8 to remove residual Triton spinning down and aspirating as before each time. Then take a pulled pipet which is very fine and remove the last of the Tris-NaCl using a rubber bulb - not suction.

   Be careful not to suck off the beads which are very fluffy, during the various washes, but be sure to suck off essentially all of the supernate on the final Tris NaCl wash.
7. Add 5 ul SDS PAGE loading buffer containing 5 ul/M DTT added just before use, to each sample. Vortex and incubate at 37øC for half an hr. You can leave the samples for several hrs at 37øC but I wouldn't do it overnight. Similarly, prepare sample of 14C markers using 1 ul of stock markers per lane of gel prepared in 10 ul loading buffer as

with the other samples.

8. Bring enough water to cover the bottom third of the tubes to a rolling boil and place the capped samples into the boiling bath for 2 to 3 mins then remove to room temp.
9. Spin 2 min in microfuge at room temp and place tubes in order to be loaded on gel.
10. Load SDS gel of appropriate percentage.

B. Immunoprecipitation of denatured proteins solubilized in SDS

1. TCA precipitate the chilled translation products by adding 2 volumes of ice cold 20% TCA, vortex, and incubate from 10 mins to several hours on ice.
2. Spin out precipitate 15 to 20 seconds in microfuge, aspirate sup., add 400 ul ice cold ethanol:ether 1:1 spin again and aspirate sup.
3. Add 1% SDS 0.1 M Tris pH 8.9 (add a volume equal to the amount of starting translation product which was TCA precipitated). Vortex, incubate at 37øC for half an hour with another vortex and incubation if precipitate hasn't dissolved by then.
4. Heat the dissolved samples in boiling bath for 2-4 minutes.
5. Dilute samples with 20 volumes of 1 X TX-SWB to take up all excess SDS into Triton micelles (final Triton conc is approx. 1%, final SDS conc is approximately .05%.)
6. Add antiserum and proceed as for previous immunoprecipitation protocol starting with step 3.

C. Preparation of samples for SDS gels directly, without immunoprecipitation

1. TCA precipitate and ethanol ether wash as above for large volumes of WG translation products (> 3 ul). For Retic translations use 1 ul total translation products.
2. Solubilize directly in SDS loading buffer (for wheat germ dilute the loading buffer by 2) with 0.5 M freshly added DTT.
3. Prepare for gels as previously.