

## DSP Crosslinking

To a concentrated protein sample (I started with a 20 mg/ml lysate), add 1-3  $\mu$ l of a 20mg/ml DSP stock (in 100% DMSO).

Incubate for 10-45 min on ice, or one can also incubate at increased temperatures if the complex is stable. I incubated on ice for 30 min.

To terminate the reaction, add 0.5 volumes of 0.4 M ammonium acetate. Incubate on ice 10 min.

Add SDS to 1% and heat to 65°C for 10 min. Cool on ice.

Add buffer containing 1% Triton X-100 (immunoprecipitation buffer) to dilute the SDS. To a 50  $\mu$ l sample I added 700  $\mu$ l of buffer.

You can next preclear the samples to decrease the resulting background as follows:

Add BSA to 0.05% (BSA in immunoprecipitation buffer)

Add Protein-A Sepharose beads

Incubate on nutator in the coldroom for 1-2 hours.

Spin in coldroom microfuge for 1 min.

Remove supernatant and place in fresh tube

Add serum for immunoprecipitation. The amount added will be variable depending on the antibody used. For Sec8 precipitation I used 8 $\mu$ g of affinity purified antibody protein (6  $\mu$ l of aff. pur. sera) For Sec4 immunoprecipitation, I used 3.5  $\mu$ l of crude Shy sera.

Incubate on nutator in the coldroom for at least 2 hours. You will have to do a test to determine the time required to obtain good precipitation. I used overnight incubations in antibody, as a 2 hr incubation resulted in only 35% the amount of precipitation as a overnight incubation.

Add Protein-A Sepharose beads (60  $\mu$ l of a 28.1 mg/ml stock in immunoprecipitation buffer) and incubate 2 hrs on the nutator at 4°C.

Spin in microfuge for 20-30 seconds. If you are doing a precipitation using a cold (non-radiolabelled) lysate save some of this first supernatant to do a Western blot to determine the percent of the total lysate that was precipitated. If the lysate was <sup>35</sup>S-labelled, discard the supernatant in the appropriate waste.

Wash the pellet with at least 4-5 washes of immunoprecipitation buffer. For 2 washes I included 2M urea in the buffer and for another 2 washes I included 500 mM NaCl in the buffer. These washes helped to reduce the background, especially when using a radiolabelled lysate.

To the final pellet, resuspend in 20-100  $\mu$ l of 1X sample buffer containing 50 mM DTT (to reduce the crosslinker and release proteins). Boil for 5 min and load onto gel. For precipitations using a cold lysate perform Western blot with nitrocellulose transfer.