Pelleting Assays

These assays are useful for determing if proteins being assayed have targeted to membranes. The translation reaction is layered on top of a sucrose cushion (1x retic,0.5M sucrose,1mM DTT,PIN) and spun at 100 000xg for 15 minutes. This can be done either in the airfuge or the TLA100, depending on the number of samples. The sucrose cushion is usually 2x the volume of the translation reaction but it can be larger. After spinning, the samples are separated into top (half of total volume), middle (other half of total volume), and bottom (pellet). The bottom fraction is resuspended in an equal volume of 0.1M Tris, 1% SDS, pH 8 and heated to 65 degrees Celsius for 15 minutes. Equal volumes of each fraction are then run on SDS-PAGE. An equivalent of 0.5 to 1ml of the original translation reaction gives best results.

Pelleting assays can also be performed in urea for targeting of molecules (such as SRa) that are resistant to extraction by denaturants.