

GLUTERALDEHYDE AND EGS CROSSLINKING

The specificity in protein/protein interactions as detected by chemical crosslinkers depends in part on the length of the crosslinkers chemical structure; the shorter the structure the tighter the interactions must be in order to obtain efficient crosslinking. Gluteraldehyde is a relatively short crosslinker and therefore has the advantage of only crosslinking proteins in close contact. It is also active under most buffer conditions, is water soluble and has a relatively low temperature dependence compared to some crosslinkers. The main disadvantage in using gluteraldehyde compared to other crosslinkers is that it is not reversible/cleavable.

The main parameter in all crosslinking experiments is the concentration of crosslinker; too little and there will be no crosslinked product, too much and everything will be crosslinked together (especially if you are crosslinking a cellular extract). Unfortunately, most crosslinkers decay (often from exposure to air/water) and therefore the appropriate concentration of crosslinker can vary with time. For this reason crosslinking experiments always employ a range of crosslinking concentrations to help insure the correct concentration is used. Typical ranges for gluteraldehyde concentrations range from 0.001% to 0.1% final. The initial experiment may include 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2% (all diluted into the sample from 10x stocks). Typical concentrations for EGS crosslinking are 0.5, 1.0 and 2.0 mM. Once the appropriate range has been determined for the particular sample (i.e. crude extract or purified protein(s)) and incubation temperature a more refined range can be utilized. Typical incubation times for a crosslinking experiment range from 10-30 minutes depending on the incubation temperature (30 min. at 4°C; 10 min. at 37°C). At the end of the incubation time the reaction should be quenched with final concentrations of 0.1-0.5M Tris + 1x Sample Buffer.

Resolution of the crosslinked products is done using a "non-stacked" SDS-PAGE Laemmli or Na-Borate (5x Na-Borate buffer = 0.5M Boric acid, 0.5M NaOAc pH8.5 and 0.5% SDS) gel. The percent gel to use will of course depend on the expected size of your products; for Hsp70 and HSF's usually a 6% gel is best.