

Affinity Proteomics – Mass Spectrometry (AP-MS)

Checking the quality of the purifications

Question (1): is the target protein (bait) visible in the eluted (purified) sample?

Suggested experiment: Western Blot analysis of the eluted (purified) sample. An additional test experiment by shotgun LC-MS/MS is beneficial (the bait may be readily visible in a Western but the amount may not be sufficient to be seen in a LC-MS/MS run).

Question (2): is the antibody eluting together with the bait?

Suggested experiment: use elution procedure with beads coupled to antibodies, and run a SDS-Gel with Coomassie staining of the eluted (blank) sample. No traces of antibody should be seen (bands at 25 and 50kDa in presence of DTT, band at 150kDa without reducing agents). Consider cross-linking if the antibody is eluted, or to use tagged bait, e.g. Flag tag eluted with Flag peptide.

Question (3): is the ratio bait/antibody correct?

Suggested experiment: Western Blot analysis of the flow-through. When a large amount of bait is found, repeat the purification increasing the amount of antibody. When the bait is not found in the flow-through, repeat the purification loading higher amount of sample.

Question (4): is the bait eluted completely?

Suggested experiment: perform multiple sequential elutions of the bait from the beads, and check them via Western Blot analysis. Does an elution under strong conditions elute further bait?

Question (5): is the bait lost during the washes?

Suggested experiment: collect the wash steps and check them via Western Blot analysis. Such samples should contain less bait than the final elution steps.

Question (6): is the purification specific?

Suggested experiment: SDS-Gel with Coomassie staining of starting material and purified sample. If the band profiles look similar, the purification is not specific.

Question (7): is the purification reproducible?

Suggested experiment: LC-MS/MS analysis. If the identification of interaction partners is based on mass spectrometry, you should ensure that the replicate experiments (ideally performed on different days) give comparable results.