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Affinity Purification Mass Spectrometry (AP-MS)

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Affinity Purification Mass Spectrometry (AP-MS) is a highly effective method for isolating and identifying binding partners to a target protein. One of the most common methods used for AP-MS experiments is expressing the target protein with a unique peptide sequence tag such as FLAG, c-Myc, HA or V5 and using the well documented high affinity antibodies to these peptide sequences. The successful use of this tagging approach is well represented in current literature. However, we have observed that the matrix used to immobilize the antibody is critical for successful affinity purification. Initial observations suggested high variability between experiments using the identical M2 Clone antibody and p53 FLAG-tagged protein. We have experimentally evaluated several different resin formats based on these reagents and identified multiple parameters underlying the observed variability in the affinity purification procedure. Additionally, crosslinking procedures, reagents, and dilution conditions with different detergent additives were found to differentially affect these resins and have critical impact on the success of the AP-MS experiment. Examples of successful AP-MS experiments after optimization of these parameters will be presented.

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