FLAG-Tag Protein Purification Protocol for Mammalian Cells

Compiled by: Hossein Davarinejad

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General Notes

The following protocol is based on and optimized for over expressed FLAG-tagged proteins from mammalian cells (U2OS) grown in one 10 cm^2 plate transfected at 90% confluence and harvested after 48 hours. This protocol works well for co-purification of interacting proteins (based on FLAG-Ring1B/Bmi1).

1 Re-suspend and Sonicate Cells

Re-suspend the frozen mammalian cell pellet in 2 mL of FLAG-Purification Suspension Buffer (1XPBS, 1mM DTT, 1X Protease Inhibitor). This re-suspension buffer does not have any lysis agents in order to preserve enzymatic activity. Purifications not harvesting for activity may use actual lysis buffers in addition to sonication (1% Triton X or other). This protocol uses sonication only.

Transfer the mixture to a clean 2 mL microtube and sonicate 2 times for 10 continuous seconds each, with 1 min pause in between using "Amplitude Setting 1 [out of 20]" on a Misonix XL-2000 sonicator.

2 Prepare and Incubate Cell Lysate with Beads

Centrifuge the cell lysate for 20 min at 12,000 g at 4° C to pellet lysate debris.

Use a 2 mL "micro" purification column and flush the column with 2mL of the FLAG-Purification Suspension Buffer.

Transfer 100 µL of M2 FLAG Affinity beads to the column and wash the gel away with 2 mL of FLAG-Purification Suspension Buffer.

Dry the nose (bottom) of the column and close it with the cap very tightly, then transfer the supernatants from the centrifuged cell lysate to the column gently avoiding the debris pellet.

Cap the column and incubate on a rotator at 4°C for 1.5 hours. This may be extended to overnight if enzymatic activity of protein is not a concern.

3 Wash The Column

Wash the column after incubation with at least 10 mL of FLAG-Purification Suspension Buffer. If the column doesn't flush by gravitational flow, centrifuge at low speed (~300-400 RPM) for just a few seconds.

4 Elution

To lute the column by incubating the beads at 4^{o} C for 30 min with 100 µL of 3X FLAG Peptide at a 100 ng/mL working concentration [the "3X" in 3X FLAG Peptide is not any indication of concentration, it simply refers to 3 tandem repeats of FLAG sequence in the peptide]. You can make a 1 mL of the working concentration by mixing 20 µL of the commercially available 5 mg/mL stock with 980 µL of TBS.

At the end of the incubation centrifuge at low speed for a few seconds if column does not elute by gravitational flow.

You may do additional elution steps to recover more protein.