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Flag co-immunoprecipitation

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Protocol status: Working

We use this protocol and it's working



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Abstract

Protocol used to pull down V5-FLAG-Gcase interacting proteins in HEK cells

Flag co-immunoprecipitation

- 1 Detach HEK cells using Accutase for 5 minutes at 37°C and collect them.
- 2 Spin cells in a centrifuge at 250g for 5 minutes at room temperature.
- 3 Remove the supernatant and wash cells in PBS.
- 4 Repeat steps 2 and 3 for a total of 2 washes.
- 5 Lyse cells in 1% TBS + 0.5% NP40 + PI/PHI (Pierce #A32959)
- 6 Filter lysates through a 0.22uM PES syringe filter (Millipore).
- 7 Wash 50ul of M2 anti-flag agarose resin (Sigma-Aldrich, #A2220) according to the manufacturer's instructions for 10 mg of total protein.
- 8 Incubate 10 mg of lysates with prewashed M2 anti-flag agarose resin for 2h at 4°C on a rotating wheel.
- 9 Elute Flag with Flag Peptide (Sigma-Aldrich, #F3290) by moving on a wheel for 20 min at 4°C followed by a spin for 1 min at 1000 g.
- 10 Collect the flow-through and proceed with TMT labeling or Western Blot analysis.