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We use this protocol and it's working

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Flag co-immunoprecipitation

In 2 collections

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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 antiflag 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.