Immunoprecipitation of Affinity Agarose

1. Gently re-suspend affinity agarose, mix evenly, and aspirate 40 μL of gel suspension into the centrifuge tube. Wash the affinity gel three times with 10 times the gel volume of 1xPBS ,centrifuge at 5000 rpm for 30 sec, discard the supernatant.

2. Add 50-200 μL eukaryotic cell lysate containing target protein and incubate for 2h in a shaker at room temperature or overnight at 4°C.

3. Wash the affinity gel three times with 10 times the gel volume of 1x PBS, centrifuge at 5000 rpm for 30 sec, discard the supernatant.

4. Wash the affinity gel with 5 times the gel volume of PBST prewashing solution precooled to 4°C to remove non-specific binding proteins. Centrifuge at 5000rpm for 30sec and discard the supernatant.

5. Add 20 μL 1x PBS and 5μL 5x loading buffer, boil for 5 min, cool to room temperature and centrifuge.

6. Take the supernatant for SDS-PAGE test and for subsequent Western Blotting detection.

Immunoprecipitation of Beads

1. Gently re-suspend magnetic beads, mix evenly, take 40 μL magnetic beads suspension, put it in the centrifuge tube, add 500 μL 1x PBS, fully suspended the suspension and place on the magnetic rack for magnetic separation. After the magnetic rack is left standing for 10 seconds, discard the supernatant. Repeat this washing step twice。

2. Add 50-200 μL eukaryotic cell lysate containing target protein, gently re-suspend magnetic beads, and incubate in a shaking table at room temperature for 2h or at 4°C overnight.

3. After standing on the magnetic rack for 10 seconds, transfer the supernatant to a new centrifuge tube for later use. Add 500 μL 1xPBS, mix gently, clean the magnetic beads, perform magnetic separation, and discard the supernatant. Repeat twice.

4. Add 20 μL 1xPBS and 5 μL 5x loading buffer, boil the sample for 5min, cool it to room temperature and centrifuge.

5. Take the supernatant for SDS-PAGE test and for subsequent Western Blotting detection.

Affinity Purification of Affinity Agarose

1. Preparation of cell lysate: Collecting cells. Re-suspend the cells with 1x PBS pre-cooled to 4°C, centrifuge at 1000 rpm for 3 min, and discard the supernatant. Repeat. Add the corresponding volume of lysis buffer L1 according to the number of cells, and place on the ice for 10-20 min after repeated blowing. Treat cell lysate with ultrasonic crusher until cell lysate is clear and no longer viscous. After 30 min on ice, centrifuge at 12000 rpm for 10 min at 4°C. Take out the supernatant and freeze at −80°C. If the target protein is secreted and expressed, the above treatment is not required, the supernatant of the medium can be directly collected and the following steps can be performed after concentration.

2. Gently re-suspend affinity agarose, mix evenly, and aspirate 2 mL of gel suspension into purification column.

3. Add 1x PBS 10 times the volume of gel to the gel column to wash the affinity gel.

4. Add the eukaryotic cell lysate containing the target protein and incubate it overnight at 4°C in a shaker.

5. Add 1x PBS of 5 times the volume of gel to wash the affinity gel that binds the target protein. Repeat this step 3 times.

6. Competitive elution: add the acid pre-washing buffer E1 5 times the volume of the precooled gel, to wash the affinity gel. Add peptide competitive eluent with the volume of 2 times of gel, incubate at 4°C in shaking table for 2 h, and collect the eluent. Identify protein purity through SDS-PAGE, and process and store protein as required. (Or select acid elution according to protein properties: refer to the instructions for detailed steps)