Protocol for GST-Fusion Protein Pulldown/Western Blotting Analysis

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Preparation of cell lysate

1, transfect cell lines in T-25 flasks with your interest gene;
2, lyse cells with IP lysis buffer-PI after 30-48hrs (T25 flask, add 2 ml buffer);
3, spin down the lysate to get the super.
4, pre-clear lysate with blank beads
  (1ml lysate : 80ul blank beads----tumble it for 1 hr @ 4C);
5, spin down to collect the super.

Pulldown

1, Pre-clear lysate (100ul) + GST-fusion protein (elusion protein, not beads) ----
incubate it @4C for 1 hr;
2, add blank beads 10ul to each reaction tube and tumble @4C for 1hr;
3, Spin down and remove the super.
4, wash the beads 3 times with IP-PI buffer;

SDS-PAGE and Transfer

1, add IP buffer 5ul and 2X loading buffer 10 ul and 2-ME 3ul ;
2, boil it for 10 mins
3, load it on SDS-PAG,
4, Run: 180V and 30mA for 1hr to 1:20 hrs;
5, transfer proteins to the membrane: 250mA + 40mins to 60mins(depends on the
MW of target protein)

Western Blotting

Blacking(90mins);
1st antibody incubation(60-90min)
wash 3 times
2nd antibody incubation(30-40min)
wash 3 times
add the ECL detection reagent mix and wait for 1 min
expose the membrane.