Protocol for Immunoprecipitation (IP)

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*Cell Lysis

Remove the mediun completely;
Add lysis buffer-PI (i.e., protease inhibitors: 50ml +1 tablet) 300ul/well (6-well plate);
Incubate on ice for 15mins with occasional rocking;
Transfer cell lysate to 1.5ml tube and spin down @ top speed in cold room;
Transfer the sup. to new tubes

*Pre-clearing

Add 30ul 50% slurry of protein G- Sepharose (pre-washed with lysis buffer) beads to cell lysate;
Incubate in cold room with end-over-end mixing for 60mins;
Spin down 1min;
Collect the super.

*IP

Add anti-HA 10ul to each lysate;
Incubate on ice for 60mins;
Add 30ul 50% slurry of PGS (pre-washed);
Incubate in cold room with end-over-end mixing for 60mins;
Isolate I-complexes bound on PGS by spining 1min in cold room;
Wash the beads 3X with 500ul lysis buffer (no-PI);
Remove the buffer as much as possible
Add 25ul 1X laemmli sample buffer and 3ul 2-ME
Boil them for 10mins.

*Run SDS-PAGE
*Western-Blot

Lysis Buffer		for 100ml
50mM Tris-HCl pH 7.5	1M	5ml
100mM NaCl	5M	2ml
0.5% NP-40 (10% stock)		10ml
0.3mM NaVO3		5.52mg
50mM Na F		210mg
20mM Na Pyrphosphate		892mg
1mM PMSF		17.42mg