

## NB500-249 Protocol

### Immunoprecipitation protocol for Beclin 1 Antibody (NB500-249)

#### Immunoprecipitation Protocol:

1. Cells in 2x 75cm flasks (60% confluency) are scraped with 0.5ml of Tris lysis Buffer (50mM Tris, 150mM NaCl, 1mM EDTA, 100ug/ml PMSF, 1% triton).
2. Lyse 1h at 4C, with gentle agitation.
3. Centrifuge to clear the lysates.
4. 0.1 ml of lysate is kept aside for Western Blot experiments.
5. IP : Add 5ul of polyclonal beclin antibody (NB 500-249) to 0.4ml of lysate (1:80 dilution).
6. Incubate overnight at 4C, with gentle agitation.
7. Next day, add 60ul of protein A sepharose beads to the lysate.
8. Incubate for one hour at 4C.
9. Wash beads 3X with Tris lysis buffer.
10. Beads are re-suspended with 15ul of Laemmli buffer and boiled.
11. A SDS-PAGE gel is run and the proteins are transferred to a membrane.
12. The efficiency of IP is determined by using a monoclonal anti-beclin antibody.