

NB400-116 Protocol

Immunoprecipitation protocol for ABCE1 Antibody (NB400-116)

ABCE1 Antibody: https://www.novusbio.com/products/abce1-antibody_nb400-116
Immunoprecipitation Procedure

1. Add 1ug rabbit IgG to 0.5 ml supernatant from HeLa or HEK293 cell line lysate*
2. Add 20 ul protein G plus/protein A-agarose beads
3. React for 1 hour @ RT in rolling oven or shaker
4. Spin down agarose beads and draw the supernatant
5. Add 1-2 ul of anti-ABCE1 (NB 400-116) to 0.5 ml of the cell lysate supernatant
6. React for 4 hours @ RT in rolling over
7. Add 50 ul protein G plus/protein A-agarose to ABCE1 solution for 1 hour in rolling oven @ RT
8. Spin down agarose beads and washed beads 3x with RIPA buffer (+protease inhibitors)
9. Add 2x 50 ul SDS loading buffer (+DTT) to the beads
10. Heat mixture for 10 minutes @ 85 degrees celcius
11. Spin reaction mixture @ 12K rpm for 5 minutes

* Cell line lysate = 75 cm flask dish culture, nearly confluent / 0.5 ml Pierce mammalian protein extraction buffer + protease inhibitors cocktail (100:1 was added for lysate) / lysate centrifuged 12K rpm for 10 min.

**The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.