Immunoprecipitation Data Form

Catalog #: NBP2-16753

Lot Number: 40499

Species: Rabbit

Cell Line/Tissue: 293 EBNA, HeLa, TK-

Subcellular Fraction:

Concentration: 2uL of Ab in 500 uL of cell lysates

Preparation: Collect cells, lyse in 1% NP40 lysis buffer with protease inhibitor, pre-clear for 1hr/4 C, incubate with antibody overnight at 4 C, add Protein G beads and incubate 2hrs/ 4 C. Wash beads with TBS. WB: Add 1:1 2x Loading buffer, boil for 5-10mins with 2-ME, run SDS-PAGE.
Controls: Click here to enter text.

PAGE Gel: 12%

PAGE: 100V/ ~100mins/ 12% polyacrylamide gel

Membrane: PVDF

Transfer Conditions: 100V/ 60min

Blocking Solution/ Duration: 5% skim milk, BSA

Immunoprecipiation:

IP Antibody Diluent/ Dilution: 2uL antibody in 500uL cell lysate
IP Antibody Incubation Time/ Temp: overnight/4 C
IP Antibody Sample Ratios Tested: Click here to enter text.
Matrix used to Precip. Ab/Ag Complex: Protein G beads

Western Blot

Primary Antibody Storage condition: -20 C
Reconstitution & Aliquot information: Click here to enter text.
Primary Antibody Diluent/ Dilution: 1:1000
Primary Antibody Incubation Time/ Temp: 1 hr/ RT, Overnight/4 C
Wash Solution Composition/Repetition/Time: 0.1% PBS-Tween 20/ 0.1% TBS-Tween 20/ From 5-10mins/ 3-5 repetitions
Secondary Antibody Diluent/Dilution: 1:1000 goat-anti-rabbit-HRP in 2% skim milk
Secondary Antibody Incubation Time/Temp: 1 hr/RT
Wash Solution Composition/ Repetitions/ Time: 0.1% PBS-Tween 20/ 0.1% TBS-Tween 20/ From 5-10mins/ 3-5 repetitions

Detection Substrate: Chemiluminescent

Development Procedure/ Time: 5mins/ RT 1:1 chemiluminescent, followed by 10min in chemi channel on Odyssey Fc

Expected MW Bands: ~58

Observed MW Bands: ~37

Additional Observations: Click here to enter text.