

NB500-201 Protocol

Immunoprecipitation Protocol Specific for Survivin Antibody (NB500-201)

Immunoprecipitation Procedure

- 1) Lyse cells plated in a 60mm dish:
 - a) 300 ul CHAPS buffer [50mM Tris-HCl, pH 7.5/50mM NaCl/1mM EDTA/1% NP-40/0.1% CHAPS/1mM NaVO₄/1mM PMSF]
 - b) Rock for 20 minutes at 4 degrees C
- 2) Harvest lysate and spin down the insoluble material at 14K rpm
- 3) Collect soluble fraction
- 4) Pre-clear lysate with 40 ul of 50:50 slurry of Protein A beads, rocking for 1 hour at 4 degrees C
- 5) Spin down beads at 2K rpm, at 4 degrees C
- 6) Collect pre-cleared lysate
- 7) Incubate lysate with 5-7ug of anti-Survivin (NB 500-201) overnight, rocking at 4 degrees C
- 8) Add 50 ul of Protein A 50:50 slurry for 2 hours, rocking at 4C
- 9) Wash beads with 200 ul of CHAPS buffer, three times
- 10) Denature immune complex by adding 2x Sample Buffer, containing 2-ME
- 11) Boil for 10 minutes and load onto an SDS-gel.