

## NB500-205 Protocol

# Protocol specific for Survivin Antibody (NB500-205)

### Western Blot Procedure

- 1) HeLa whole cell lysates were heated to 70 degrees C for 10 minutes and then microfuged at room temperature.
- 2) 65 mg of lysate were electrophoresed (200 V) through a 4-12% NuPAGE Bis-Tris gel, for 1 hr.
- 3) Proteins were transferred (30 V) onto a nitrocellulose membrane for 40 minutes.
- 4) The membrane was incubated for 1 hr. at ~ 27 degrees C (RT) in blocking buffer [TBST / 5% nonfat milk].
- 5) Rinsed the membrane twice in TBS.
- 6) Incubated the membrane with a dilution of (NB500-205) anti-Survivin antibody, diluted in TBS + 1% BSA, overnight at 4degrees C, gently shaking.
- 7) Washed the membrane in TBST; 1x15 min., 3x5min.
- 8) Incubated the membrane with a goat anti-mouse secondary antibody, diluted in TBS + 1% BSA, gently shaking for 35 minutes at room temperature.
- 9) Washed the membrane in TBST; 1x15 min., 3x5min.
- 10) Visualized membrane by ECL (AlphaInnotech ).

NOTE: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.