

## NB500-201 Protocol

### Western Blot protocol for Survivin Antibody (NB500-201)

[[URL: [https://www.novusbio.com/products/survivin-antibody\\_nb500-201](https://www.novusbio.com/products/survivin-antibody_nb500-201)]][[Caption:Survivin Antibody]]

#### Western Blot Procedure

- 1) Cells were pelleted, washed in 1XPBS, suspended in ice water (~ 5 x 10<sup>6</sup> cells/ml), and placed on ice
- 2) Lysates were prepared with the addition of 2X lysis buffer [2% SDS/ 50mM Tris-HCl / 10% glycerol]
- 3) Lysates were heated to 95 degrees C for 3 minutes and then microfuged at room temperature for 10 minutes
- 4) 50 ug of lysate were electrophoresed (150 V) through a 4-15% PAGE
- 5) Proteins were transferred (60 V) onto an Immobilon-P membrane (Millipore Corp.) for 45 minutes
- 6) The blot was blocked overnight at 4 degrees C in blocking buffer [1XPBS, pH 7 / 5% nonfat milk / 0.1% Tween-20]
- 7) Washed the blot in 1XPBS / 0.1% Tween-20
- 8) Incubated the blot with 1 ug/ml of (NB500-201) anti-Survivin antibody, diluted in blocking buffer, for 2 hours at room temperature
- 9) Washed the blot in 1XPBS / 0.1% Tween-20
- 10) Reacted the blot with HRP-conjugated donkey anti-rabbit Ig, diluted in 1XPBS / 0.1% Tween-20, for 30 minutes at room temperature
- 11) Washed the blot in 1XPBS / 0.1% Tween-20
- 12) Visualized blot by ECL and autoradiography

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