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NB500-201 Protocol

Western Blot Protocol Specific for Survivin Antibody (NB500-201)

Western Blot Procedure
1) Cells were pelleted, washed in 1XPBS, suspended in ice water (~ 5 x 10(6) 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.