Protocol specific for Caspase 7 Antibody (NB500-206)

Procedure Guide for NB 500-206 Monoclonal anti-Caspase 7

Western Protocol

1) Prepare samples for a NuPAGE gel and heat them at 70 degree Celsius for 10 minutes.
2) Run a 4-12% NuPAGE Bis-Tris gel, loading 50-100 mg of whole cell extract per lane, for 45 minutes at 200 Volts.
3) Soak transfer sponges and gel 15 minutes and nitrocellulose membrane 5 minutes prior to transfer.
4) Transfer the proteins to the nitrocellulose membrane for 1 hour at 30 Volts, in 4 degrees C transfer buffer.
5) Block the membrane in TBST + 5% non-fat dry milk, for 1 hour at room temperature (RT), shaking gently.
6) Rinse the membrane twice with TBST.
7) Incubate the membrane in anti-Caspase 7 [NB 500-206], diluted 1:500-1:1,000 in TBS + 1% BSA, for 90 minutes at RT.
8) Wash the membrane once for 15 minutes, then 3 times 5 minutes, in TBST at RT.
9) Incubate the membrane in anti-mouse conjugated to HRP (secondary antibody), diluted in TBS + 1% BSA for 35 minutes at RT.
10) Wash the membrane once for 15 minutes, then 3 times 5 minutes, in TBST at RT.
11) Develop membrane in chemiluminescent reagents, as instructed by kit-vendor.

*Positive control(s): HeLa whole cell extracts (cat# NB 800-PC1)  
Jurkat whole cell extracts (cat# NB 800-PC2)