

NB500-207 Protocol

Protocol specific for Caf1p150 Antibody (NB500-207)

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

Western Protocol

- 1) Prepare samples for a NuPAGE gel and heat them at 70 degrees C 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)