

## NB500-210 Protocol

### Western Blot Protocol for Caspase 3 Antibody (NB500-210)

[[URL:[https://www.novusbio.com/products/caspase-3-antibody-cpp32-4-1-18\\_nb500-210](https://www.novusbio.com/products/caspase-3-antibody-cpp32-4-1-18_nb500-210)]][[Caption:Caspase-3 Antibody ]]

Procedure Guide for NB 500-210 Monoclonal anti-Caspase 3

#### Western Protocol

- 1) Run a 10-15% SDS polyacrylamide gel, loading ~20 ug of cell extract per lane.
- 2) Transfer the proteins to a membrane.
- 3) Block the membrane in PT-T20 [20 mM Tris-HCl, pH 7.4 / 150 mM NaCl/0.5% Tween 20] + 5% NFDM [non-fat dry milk], for 3 hour at room temperature (RT), shaking gently.
- 4) Rinse the membrane twice with PT-T20.
- 5) Incubate the membrane in anti-Caspase 3 [NB 500-210], diluted 1:500-1:1,000 in PT-T20 + 5% NFDM, for 60 minutes at RT.
- 6) Wash the membrane for 15 minutes, 3 times, in PT-T20 at RT.
- 7) Incubate the membrane in anti-mouse conjugated to HRP (secondary antibody), diluted in PT-T20 + 5% NFDM for 60 minutes at RT.
- 8) Wash the membrane for 15 minutes, 3 times, in PT-T20 at RT.
- 9) Develop membrane in chemiluminescent reagents, as instructed by kit-vendor.

\*Positive control(s): Human kidney 293 cells

#### To Activate the Cell Extracts:

Bring the extract to a final concentration of 5 mM dATP.

Incubate the extracts at 37C for 15-30 minutes.