

NB100-439 Protocol

Western Blot protocol for Separase Antibody (NB100-439)

Separase Antibody (XJ11-1B12): https://www.novusbio.com/products/separase-antibody-xj11-1b12_nb100-439

1. Load protein on gel (ie: ~15 ug of Ecdysone-inducible cell line 5C6) and run.
2. Transfer protein to nitrocellulose (Schleicher&Schuell, cat# BA83).
3. Block the membrane with 5% milk for 30 minutes at room temperature.
4. Incubate the membrane with anti-separase [cat# NB 100-439] (1:500), overnight at 4C or 2 h at room temperature. Milk or PBS-T are good for this dilution.
5. Wash the membrane 3 times, 10 minutes per wash in PBS with 0.1% Tween-20 (PBS-T).
6. Incubate with secondary antibody for 30 minutes at room temperature. Use milk in PBS-T as a diluent.
7. Rinse the membrane 2 times with deionized water and place in an ECL working solution.
8. Expose to the film.