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## NB100-407 Protocol

### Western Blot protocol specific for 14-3-3 gamma Antibody (NB100-407)

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1. Load protein on gel (ie: ~30 ug of HeLa whole cell control lysate) 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-14-3-3 gamma [cat# NB 100-407] (1:1,000), 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.