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NB100-103 Protocol

Western Blot protocol for Ku80 Antibody (NB100-103)

Procedure Guide for NB 100-103 Western Blot
1. Proteins are separated on a 12% SDS/PAGE gel at 175V for 50 minutes. 2. Proteins are transferred to PVDF for 1.5 hours at 6V. 3. Following the protein transfer, the filters are blocked with 5% NFDM in PBS-T for 1.5 hours at room temperature, gently shaking. 4. Anti-Ku85 [NB 100-103] is diluted 1:500 in dilution buffer [1% BSA in PBS] and incubated with the filters for 1.5 hours at room temperature, gently shaking. 5. The filters are then washed 1x 15 minutes and then 3x5 minutes in wash buffer [PBS-T] at room temperature, gently shaking. 6. Secondary antibody [HRP conjugated goat anti-rabbit] is diluted in dilution buffer and incubated with the filters for 30 minutes at room temperature, gently shaking. 7. The filters are then washed 1x 15 minutes and then 3x5 minutes in wash buffer [PBS-T] at room temperature, gently shaking. 8. Western is developed using Pierce SuperSignal West Dura Extended Chemiluminecensce reagent. NOTE: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.