

Western Blot Analysis

Cells were washed twice in PBS and harvested with lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, 1 mM Na₃VO₄, 1 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride) on ice for 10 min. The supernatants were collected after centrifugation at 12,000 g for 30 min. The protein concentrations were determined using the BCA protein assay kit (Pierce, Rockford, IL). Equal amounts of protein lysates were separated by SDS-PAGE and transferred onto nitrocellulose membranes, followed by incubation with optimized dilutions of primary antibody (1/1000) at 4°C overnight. After washing, the membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 1 hour. Proteins were detected with the enhanced chemiluminescence (ECL) kit (Pierce, Rockford, IL).