A review for PKR antibody (NBP1-77266)

Hepatocytes were washed twice in PBS and lysed with lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, 1 mM Na3VO4, 1μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride (PMSF) on ice for 10 min. The supernatants were collected after centrifugation at 12000 g for 30 minutes. 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 incubating with primary antibody HMGB1 (1/1000 dilution) at 4°C overnight. After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 hour. Proteins were detected with the enhanced chemiluminescence (ECL) kit (Pierce, Rockford, IL). β-actin or α-tubulin (Sigma, St Louis, MO) was used as loading control.