

Western blot analysis

Xiao Bing Tang,Li Huan,Jin Zhang,Wei Lin Wang,Zheng Wei Yuan,Yu Zuo Bai

Abstract

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Protocol

Step 1.

Protein extracts (50µg) were heated at 100°C for 5 min.

Step 2.

Protein content is evaluated by bicinchoninic acid assay (BCA Protein assay kit by Thermo Fisher) in a spectrophotometer.

Step 3.

Protein samples were separated via sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, Mass., USA).

Step 4.

PVDF membrane is incubated in blocked with 5% fat-free milk in Tris-buffered saline (2h, room temperature). PVDF Membrane is washed in T-TBS, 2 times, 10 min each.

Step 5.

PVDF Membrane is incubated overnight at 4°C in primary antibody. PVDF Membrane is washed in T-TBS, 2 times, 10 min each.

Step 6.

PVDF Membrane is incubated with the secondary antibody for 2 hr at room temperature. PVDF Membrane is washed in T-TBS, 2 times, 10 min each.

Step 7.

Membranes were developed by using a chemiluminescent substrate kit (Pierce, Pierce, Rockford, Ill., USA).

Step 8.

Densitometric values were analyzed by using the ECL Plus detection system (Millipore, Billerica, Mass., USA).