Western blot analysis

Shuangshuang Li

Abstract

Citation: Shuangshuang Li Western blot analysis. **protocols.io**
dx.doi.org/10.17504/protocols.io.h4pb8vn
Published: 04 Jun 2018

Protocol

Step 1.

Total protein was extracted from cells using RIPA lysis buffer. Lysates were quantified spectrophotometrically using nanodrop2000 (Thermo).

Step 2.

Whole-cell lysates were separated on SDS-PAGE gels, and the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA).

Step 3.

The membranes were incubated at 4°C overnight with one of the following primary antibodies: affinity purified rabbit monoclonal anti-RGS6 (1:800; Abcam Inc., Cambridge, MA, USA); anti-DNMT1 (1:1000; Abcam); anti-Nanog (1:500; Abcam); anti-Oct4 (1:500; CST Co. Inc., USA); or rabbit anti-human β-actin (1:2000; Sizhengbo, Beijing, China).

Step 4.

The membranes were then rinsed with PBS and incubated with a horseradish peroxidase-conjugated anti-rabbit antibody (1:3000; Sizhengbo). The blots were visualized using ECL reagent (Bio-rad, USA).

Step 5.

mage acquisition and analysis of band density were performed using a G:BOX (Synoptics Group, England, UK) chemiluminescence imager and GeneSys automatic control software.