Protocol

General Protocol for Western Blot Analysis

OVERVIEW

The following procedure is a general Western blot protocol for detecting specific proteins.

WESTERN BLOT PROCEDURE

1. Resolve 50 to 100 µg of a protein sample and an appropriate protein marker by gel electrophoresis.
2. Transfer proteins from the gel to a suitable membrane (i.e., nitrocellulose or PVDF).
3. Place the membrane in a clean covered dish or heat-sealable plastic bag and block with an appropriate blocking buffer for 60 minutes at room temperature using an orbital shaker or rocking platform. Decant the solution.
4. Dilute the primary antibody in blocking buffer and add to the membrane.
5. Incubate at 4°C overnight on the shaker platform. Decant the solution.
6. Wash the membrane 3 times with an appropriate wash buffer for 5 minutes at room temperature on the shaker platform. Decant the solution.
7. Dilute the secondary antibody (e.g. horseradish peroxidase) in blocking buffer and add to the membrane.
8. Incubate for 45 minutes at room temperature on the shaker platform. Decant the solution.
9. Wash the membrane 5 times with wash buffer for 5 minutes at room temperature on the shaker platform. Decant the solution.
10. Incubate the membrane with a chemiluminescent substrate for 1 to 5 minutes at room temperature on the shaker platform. Decant the solution.
11. Develop the membrane according to manufacturer's instructions.

GP_Western_Blot_V1 November 1, 2010

Stemgent
One Kendall Square, B6201
Cambridge, MA 02139
Technical Support: 1.877.783.6911
www.stemgent.com