WESTERN-BLOTTING PROTOCOL

Electrophoresis and transfer

Separate proteins by gel electrophoresis according to manufacturer's specifications.
Run standard SDS-Polyacrylamide gel and electrophoreses in a 0.5-1.5 mm thick gel.

Transfer proteins from the gel to a nitrocellulose or other membrane such as PVDF by electro-blotting according to the manufacturer's protocols.

Blocking

Block remaining hydrophobic binding sites on the membrane by incubating membrane in a blocking solution (5% non-fat dried milk, 0.05% Tween 20 in phosphate buffer solution for 30-60 minutes at room temperature or overnight at 4°C.

Primary Antibody Incubation

Dilute the primary antibody in the blocking buffer. Optimal antibody concentration should be determined by titration (a concentration of 1-2.0 µg/ml is generally acceptable). Incubate for 1 hour at room temperature or overnight at 4°C with gentle agitation.
Wash membrane three times for 5 minutes each in washing buffer (phosphate buffer saline plus 0.05% Tween 20).

Secondary antibody incubation

Incubate the membrane with appropriate conjugated secondary antibody: alkaline phosphatase or horseradish peroxydase conjugated antibody. Incubate the membrane with conjugated secondary antibody diluted in blocking buffer for 30-60 minutes at room temperature with gentle agitation.
Wash membrane three times for 5 minutes each in washing buffer (phosphate buffered saline plus 0.05% Tween 20).

Visualisation

Incubate the membrane with appropriate substrate solution and incubate for time recommended by manufacturer to visualise protein bands.

Solutions

Blocking solution
5% (w/v) non-fat dried milk, 0.05% (v/v) Tween 20 in phosphate buffer solution

Washing buffer
Phosphate buffered saline plus 0.05% (v/v) Tween 20

DIACLONE SAS - 6 Rue Docteur Jean-François-Xavier Girod - BP 1985- 25020 BESANCON Cedex - France
Tel: +33(0)3.81.41.38.38 - Fax: +33(0)3.81.41.36.36
info@diaclone.com - www.diaclone.com