www.tag-tools.de

tag-tools GmbH

Oberdorfstr. 16B
78465 Konstanz

mail@tag-tools.de
www.tag-tools.de

Western Blotting

Materials

Western Transfer Buffer:
6.0 g Tris Base
28.8 g glycine
dissolve in 1 liter H_2O
430 ml methanol
(degas after adding the methanol)
add 10 ml SDS (20%)
fill up to 2 liter with H_2O

10 x TBS (500 mM Tris / 1.5 M NaCl):
121 g Tris Base
175 g sodium chloride
dissolve in 1.5 liter H_2O
adjust pH to 7.5 with HCl
fill up to 2 liter with H_2O

TBS-T:
200 ml 10x TBS
10 ml 10% Tween
fill up to 2 liter with H_2O

Blocking solution:
TBS-T
2% BSA
0.05% NaN_3

1st antibody
***tag*-tools** antibody;
different dilutions should
be tested: 1:500 – 1:2,000

2nd antibody
HRP-labelled secondary antibody
directed against 1st antibody,
different dilutions should
be tested: 1:5,000 – 1:20,000

ECL reagents

Staining solution:
dissolve 300 mg
Coomassie Brilliant Blue
in 250 ml isopropanol
and 100 ml acetic acid
fill up to 1 liter with 650 ml H_2O

Destaining solution:
10% (v/v) isopropanol
10% (v/v) acetic acid in water

Procedure

1. Proteins in whole cell lysates are separated by SDS-PAGE (8 – 15% polyacrylamide, depending on the protein of interest).
2. After separation, the proteins are transferred from the gel to a PVDF membrane by electrophoretic transfer in a Western blotting chamber or a semi-dry transfer apparatus.
3. After transfer (1h – over night, depending on your western transfer system), the PVDF membrane is removed and incubated for 15 minutes in staining solution on a rocking platform at room temperature.
4. Incubate the Coomassie stained membrane in destaining solution until the background is washed out and protein bands appear. Now is a good time point to mark the bands of the molecular weight standard on the membrane.
5. Wash membrane twice in water, then once in TBS-T
6. Incubate membrane in Blocking solution for 1 h at room temperature or over-night at 4°C
7. Add first antibody (diluted 1:500 to 1:2,000 in Blocking solution) in a volume that covers your membrane and incubate over night at 4°C on a rocking platform
8. Wash the membrane three times for 15 min each with TBS-T at room temperature.
9. Add secondary antibody (diluted 1:5,000 to 1:20,000 in TBS-T) in a volume that covers your membrane and incubate for 1 – 2 h at room temperature on a rocking platform
10. Wash membrane three times for 15 min each with TBS-T at room temperature.
11. Incubate membrane in ECL reagents and detect the luminescence signal by X-ray film or digital ECL documentation system.