Westernblot

20X MES SDS Buffer:

195.2g MES (4-morpholineethane sulfonic acid)
121.2g Tris Base
20g SDS
6g EDTA
dH_2O to 1L

10X Running Buffer:

151g Tris base
720g Glycine
dH_2O to 5L

1X SDS-Running Buffer:

10 mL 10% SDS
100 mL 10X Running Buffer
dH_2O 900 mL

Transfer Buffer:

250 mL 1X SDS-Running Buffer
200 mL Methanol
550 mL dH_2O

PBS Buffer:

8g NaCl
0.2g KCl
1.44g Na_2HPO_4
0.24g KH_2PO_4
800 mL dH_2O
pH to 7.4 with HCl
Add dH_2O to 1L

Solution I

0.3% Tween 20 in PBS buffer.

Solution II

0.05% Tween 20 in PBS buffer

The amount of Tween-20 added to PBS should be determined for each application. Some antibodies will work fine with as low as 0.01% Tween-20, but background levels can be reduced and even removed completely with concentrations of up to 1%

Procedure:

Run SDS-PAGE gel. Make 2L of 1X Transfer Buffer. Soak gel in dH_20 for 10 minutes to remove SDS.

Cut PVDF membrane same size as filter paper and wet with methanol. PVDF membrane should never be exposed to water alone. Soak sponge, filter paper and PVDF membrane in 1X Transfer Buffer for 5 minutes.

Prepare transfer gel by putting ice bucket behind transfer box. Put gel box inside small autoclave bin filled with ice.

Clear (+) | sponge | filter paper | PVDF | gel | filter paper | sponge | black (-)

Run the gel transfer for 1.5 hours at 100V. Check amps to make sure it's running. When finished running, make sure dye is on membrane to know transfer worked.

Soak the blot in about 40 ml of 3% Blotto (3g of dry milk powder in 100mL of solution I) for 1 hour at 37^0C or overnight on shaker at 4^0C.

Wash the blot with 10 ml of Solution II twice for 5min at RT.

Dilute 150 µl primary antibody in 15 ml Solution II. Incubate the blot with primary antibody for 1 hour with shaking at RT. Save solution for possible reuse.

Wash with 10 ml of Solution II twice for 5min at RT.

Dilute 2 µl of secondary antibody (rabbit) in 10 ml of Solution II. Incubate the blot with the secondary antibody for 1 hour with shaking at RT.

Wash for 15min with 10 ml of Solution I, wash 3X for 10min with Solution II.

Spread blot on plastic wrap. Incubate the blot on the plastic wrap in ECL (500 µl Reagent I and 500 µl Reagent II) for 1 minute. Wrap blot completely with no bubbles in plastic wrap.

Scan the blot or use film in the darkroom. Only use half piece of film and expose for less than a minute.