Probing of membranes with HRP

Description

Incubation

1. In order to hydrate all peptide treat the membrane in 100% methanol for 10 minutes while shaking.
2. Washing the membrane with TBS for 3 times, each time for 5 minutes.
3. Block unspecific binding sites with blocking buffer at room temp for 2 hours or overnight.
4. Wash the membrane once in TBS-T for 5 minutes.
5. Dilute protein sample solution to a concentration of 1 to 0.1 µg/ml (depending on the expected affinity) in blocking buffer and incubate for minimum 2 hours at room temp or overnight at 4°C. For probing with peptides it might be necessary to use a concentration of up to 20 µg/ml.
6. Wash the membranes 3 times for 5 minutes each with TBS-T.
7. Incubate the membrane for minimum 2 hours at room temp or overnight at 4°C with the HRP labeled second antibody. (0.1 µg/ml in blocking buffer)
8. Wash the membranes three times for 5 minutes each with TBS-T followed by wash once in TBS.

Detection with chemiluminescence

1. Remove excessive buffer from the membranes by placing white tissue paper on the membrane. Do not use recycled tissue or paper towel which may generate fluorescence. Avoid drying of the membrane. Do not wipe or press the tissue on the membrane.
2. Wet the membrane with a thin layer of chemiluminescence (ECL) detection reagent mixture according to the instructions of the manufacturer or treat it with staining reagents.
3. For the chemiluminescence detection use a luminescence detector or, treat X-ray films.

Detection by staining

1. Preparation of development solution I (DS I) for a total of 10 ml staining solution: Dissolve 100 mg NaCl in 2.5 mL 200mMTris-HCl pH 7.4 (24.2 g/L) and 5.8mL H_2O
   This solution can be prepared in advance and stored in the fridge
2. Preparation of development solution II (DS II) for a total of 10 ml staining solution: Dissolve 5 mg 4-chloro-1-naphthol in 1.7mL methanol
   Important! This solution must be prepared always fresh shortly before use!
3. Wash the membrane another two times with TBS for 5 minutes
4. Mix DS I with DS II and add 5 µL 30% H_2O_2

5. Treat the membrane with that mixture until the staining is sufficient strong. If necessary, renew the staining solution.
6. Stop the staining process by washing thoroughly with water.

Buffers

1. TBS

Reagents	1x TBS in 1 L	10x TBS in 2 L
50 mM Tris-base	= 6 g	= 120 g
27 mM KCl	= 200 mg	= 4 g
136 mM NaCl	= 8 g	= 160 g

in distilled/deionized water
adjustment to pH = 8 with NaOH and HCl solutions

2. TBS-T

TBS + 0.05% Tween 20	= 500 µl in 1 L TBS

3. Blocking buffer

Reagents	in 100 ml TBS-T
5% Sucrose	= 5 g
4% Skim milk or Casein (or BSA)	= 4 g