

Western Blotting

Aims of the experiment

Different purification tags are commonly used to isolate fusion proteins or to detect certain proteins within lysates or other protein mixtures. Western Blotting is a technique used to identify small amounts of tagged proteins. Using a primary antibody to detect the specific target or protein present in the sample and a secondary antibody specifically detecting the bound primary antibody, Western blots are a very common tool to determine the presence of the target proteins within a sample.

Materials

- Anti-6xHis Antibody (mouse)
- Anti-mouse Alexa 680 Antibodyconjugat (donkey)
- Washing buffer (1xPBS, 0.1% Tween-20)
- Transfer Buffer
- PVDF blotting membrane
- Blocking Buffer (5% BSA)
- Filter paper
- Methanol

Procedure

1. Soak filter paper in transfer buffer and place 3 layers of wetted filter paper on cathode of a semi-dry blotter.

25 mM Tris

190 mM glycine

20% methanol

2. Place SDS-Page on top

3. Activate PVDF membrane by putting in methanol for 5-10 s and wash it afterwards in transfer buffer
4. Place membrane on top of SDS-Page and avoid bubble between the layers
5. Put another 3 layers of soaked filter papers on top and remove any bubbles within the blot-sandwich by using a roller
6. Put the top of the sem-dry blotter with anode on top and apply 350 mA for 1h, while cooling the system with an ice box on top
7. After transfer put the membrane into blocking buffer for 1 h with mild agitation
 - 5% BSA
 - 1xPBS
8. Wash the membrane 3x10 min in washing buffer with mild agitation
 - 0.1% Tween-20
 - 1xPBS
9. Apply primary antibody (in 1xPBS) and incubate at 4°C overnight with mild agitation
10. Wash the membrane 3x10 min in washing buffer with mild agitation
11. Apply secondary antibody (in 1xPBS) and incubate at RT for 1 h
12. Screen for fluorescence using an adequate device (laser scanner)