

Western Blot Protocol

- run SDS-PAGE and perform Western Blot by standard procedure
- stain membran with Ponceau to validate blotting efficiency

- block membran in 10% milk powder or 5% BSA dissolved in PBST for 1h at room temperature
- incubate with primary antibodies diluted in 5% milk powder/PBST or 2.5% BSA/PBST for 1h at room temperature or o/n at 4°C
- wash 3 times with PBST
- incubate with corresponding secondary antibodies¹ coupled to IRdye680 or IRdye800 diluted in 5% milk powder/PBST or 2.5% BSA/PBST for 1h at room temperature
- wash at least 3 times with PBST
- scan membran with LiCOR Odyssey Scanner

- membranes can be easily stripped and reprobed using LI-COR stripping solution

¹ required **secondary antibodies** can be found here:

https://licor.secure.force.com/catalog/LI_ProductListMain?categoryId=a0d60000000T9QMAA0&g=REU=&store=bio

or visit the distributor's homepage:

<http://www.licor.com/>

1x PBST

400 mM NaCl

2.6 mM KCl

1.5 mM KH₂PO₄

8 mM Na₂HPO₄

0.1% TWEEN

ph 7.4