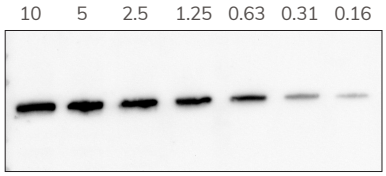
Tip Sheet

Azure Chemiluminescent Western Demo Kit

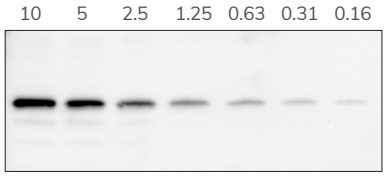
AC2212 (Anti-Rabbit) and AC2213 (Anti-Mouse)

The Chemiluminescent Western Demo Kit provides all the reagents you need to conduct a wet transfer followed by a one hour Western. The kit includes a control lysate and primary antibody which should be used, without exception. If a customer would like to use their own primary antibody and samples they may. There are sufficient reagents to perform two Western blots, below are examples of how to load your gel to accommodate the customer's needs. If you decide to evaluate experimental samples, pipette a MW Marker in the middle of the gel to separate the control and experimental samples so that it is easy to visualize where to cut the membrane.

Control Westerns

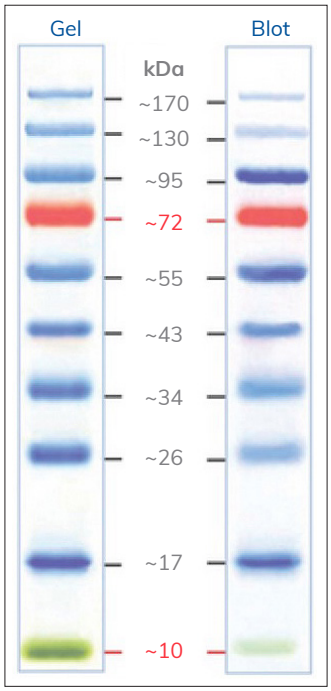


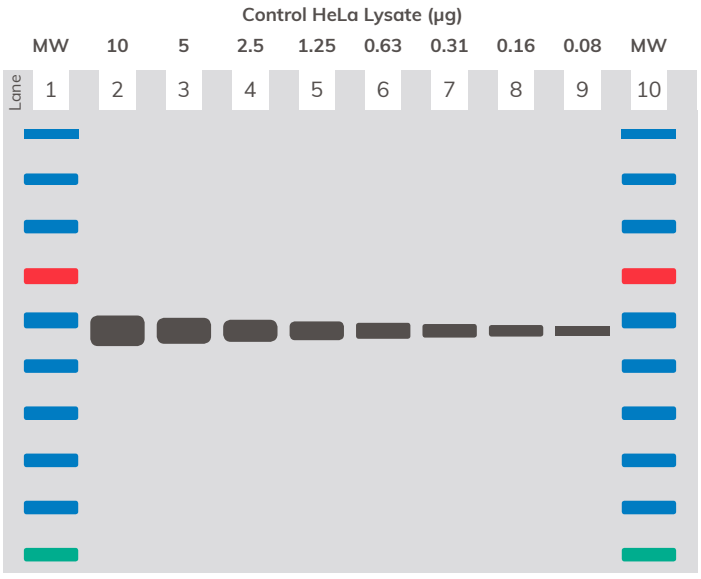
Mouse Anti-Alpha Tubulin
Control HeLa Lysate (µg)



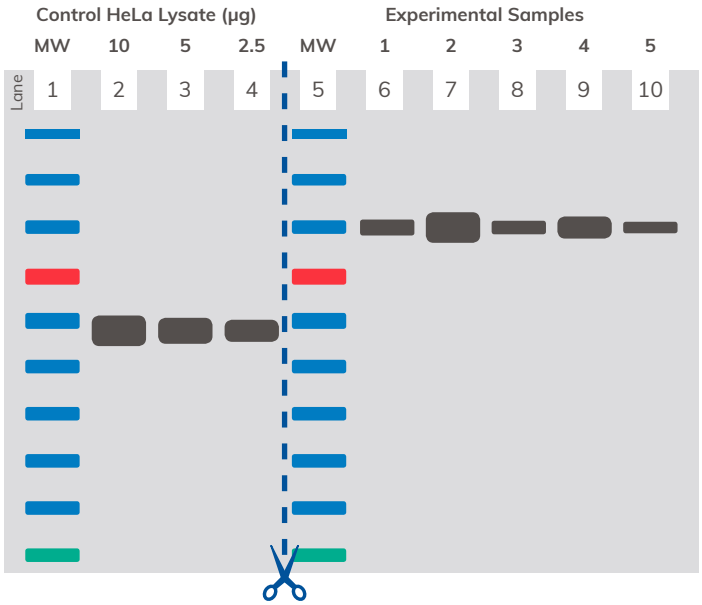
Rabbit Anti-Cyclophilin B
Control HeLa Lysate (µg)

MW Marker



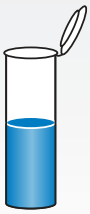


Example 1. Evaluation of Control Samples

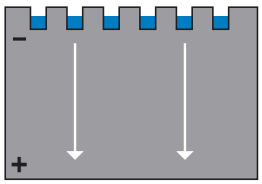


Example 2. Evaluation of Control and Experimental Samples

Workflow

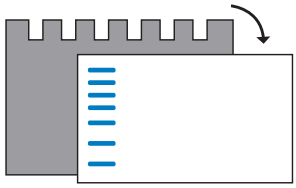


Step 1
Sample preparation

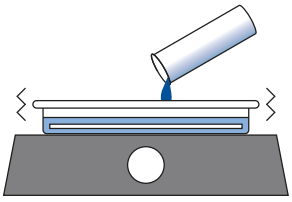


Step 2
Perform electrophoresis
Protein separation by SDS PAGE: Run the electrophoresis using your standard lab conditions, until the dye front reaches the bottom of the gel.

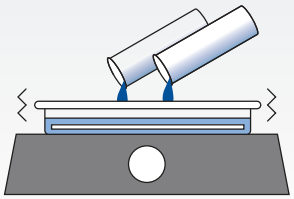
Step 3
Buffer preparation



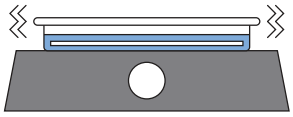
Step 4
Equilibriate membrane and transfer



Step 5
Dispense 10mL of Buffer C into incubation tray.
Incubate membrane for 10 minutes at RT with gentle agitation.

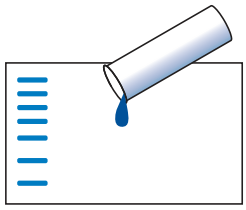


Step 6
Add primary antibody and Buffer D.
Incubate for 30 minutes minutes at RT with gentle agitation.



Step 7
Wash membrane
1X for 15 minutes with 25mL of Buffer E with gentle agitation.

Step 8
Prepare Radiance Substrate
Combine Radiance components 1:1 in sufficient amounts to obtain 0.1 mL/cm^2 of your membrane.



Step 9
Apply Radiance Substrate
Incubate for 5 minutes.

Step 10
Image blot

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