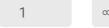


## Mar 04, 2022

## Western Blot

## Haley Geertsma<sup>1</sup>

<sup>1</sup>University of Ottawa



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This protocol is used to western blot proteins-of-interest.

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https://dx.doi.org/10.17504/protocols.io.b5wkq7cw	
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- 1 Add 4X Laemmli buffer to protein samples and incubate at 95°C for 7 minutes.
- 7m
- 2 Load samples into 12% polyacrylamide gel and run in running buffer at 80-100V for 60-120 <sup>2h</sup> minutes.
- 3 Transfer gel to 0.2μm nitrocellulose membrane at 350mA for 60 minutes at 4°C.
- 4 Wash membrane with 1X TBS-T then block in 10% milk for 30 minutes at room temperature.



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5	Wash membrane with 1X TBS-T for 4x 5 minutes.	20m
6	Incubate membrane in primary antibody solution overnight at 4°C.  Antibodies diluted to the appropriate concentration in 2% bovine serum albumin in 1X with 0.02% sodium azide	1d TBS-T
7	Wash membrane with 1X TBS-T for 4x 5 minutes.	20m
8	Incubate membrane in secondary antibody solution for 1 hour at room temperature. Secondary antibody (horseradish peroxidase conjugated) is diluted in 10% milk.	1h
9	Wash membrane with 1X TBS-T for 4x 5 minutes.	20m
10	Rinse ECL Clarity Solution over membrane 20x then image.	1h
11	Once imaging is complete, wash membrane with 1X TBS-T x2 then H <sub>2</sub> O x2 then let dry temperature overnight before storing.	10m / at room