Easy worm western blot using the iBlot:

1. Label a 1.5 ml eppendorf tube (eppi) and fill it with 10 µl H_2O
2. Remove bacteria stuck on the worms, by transferring them onto a blank NGM agar plate (without bacterial lawn). Let them crawl around for 5-10 minutes.
   Why? Less bacterial protein → cleaner blot
3. Pick 40 clean (adult) worms into the water containing eppi.
4. Add Protein Sample Buffer (Laemmli) and fill with H_2O to final volume of 20 µl.
5. Boil mixture at 95°C for 5 minutes.
6. Quickly spin eppi at full speed in table-top centrifuge for 1 minute.
   Why? Sediment viscous undissolved worm material to the bottom, so you don't load this onto your protein gel → gel runs smoothly
7. Prepare gel chamber + gels
8. Load worm mix onto gel
9. Run the gel. Settings for running depend on protein size, charge, gel chamber and gel used.
10. Equilibrate the gel in transfer buffer prior to western blotting procedure for 10 minutes on a shaker to remove excessive SDS from the gel.
    Why? The presence of SDS contained in the gel running buffer can lower the transfer efficiency of the protein during western blot procedure. This is particularly detrimental if you use dry or semi dry blotting procedures, like the iBlot system, in which the SDS is not diluted into large amounts of transfer buffer.
11. Blot the proteins using using the iBlot system. (Follow provided assemble procedure)
    Typical settings are: Program P2, 5-8 minutes
    Note: Transfer time strongly depends on size and charge of the protein. Test it!
    ~5 minutes for small / charged proteins
    ~8 minutes for large / uncharged proteins
12. Block the membrane in blocking buffer on a shaker for ~1h.
    Note: 3% Milk blocks nicely, but should be avoided if phospho-antibodies are used for protein detection, since milk contains highly phosphorylated casein. Block with 3% BSA instead.
13. Wash off excess blocking buffer by washing and exchanging PBS 3 times.
    Note: This is a dilution reaction. You can also use PBS-T(0.1%) but remember that adding detergent can also remove the blocking reagent, so be careful to not wash too long.
14. Add primary antibody (AB) in PBS-T + 2% BSA and incubate 1-3h at room temp. or o/n at 4°C.
    Note: Supposedly specificity of the AB increases at 4°C over room temp., but requires more time for binding, so incubate o/n.
15. Wash off excess AB using PBS-T 3 times.
16. Detect your primary antibody via a secondary HRP-couple antibody, fluorescent tagged secondary AB or any other preferred detection method.

Protein detection using chemiluminescence (HRP + ECL + Film):

17. For chemiluminescent detection add a HRP-couple secondary antibody and incubate for 1 h at room temp.
18. Wash off excess AB and transfer membrane into a fresh jar.
19. Add 1:1 mix of ECL or any other chemical detection solution. Usually 1-2 ml total volume is sufficient to wet a typical membrane.
20. Incubate membrane with solution mix for 1 minute and put membrane into detection chamber.
21. Cover membrane with plastic foil to prevent contact between ECL and film.
22. Place film onto plastic covered membrane in development room for 1-2 minutes (depends on the detection solution used). Using standard ECL, 40 worms material, standard film, and our standard primary / secondary worm antibodies 1-2 minutes exposure is usually to detect protein / western marker

Solutions:

PBS-T (1x):
- 1x PBS
- 0.1% Tween 20

Blocking solution:
- PBS-T
- 3-5% milk

Antibody solution:
- PBS-T
- 3-5% milk
- Primary AB: Typically 1:500 to 5.000 diluted
- Secondary AB: 1:30.000 diluted

Transfer Buffer (1x):
- 25 mM Tris
- 192 mM Glycine
- 0.1% SDS
- 10% Methanol (add fresh)

Protocols – Hymanlab by Oliver Wüseke