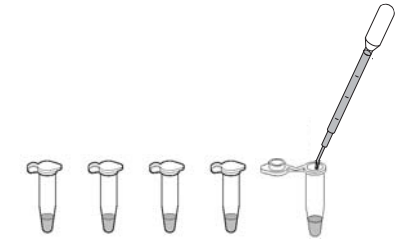
Comparative Proteomics Kit II: Western Blot Module – Quick Guide

Lesson 1 Quick Guide



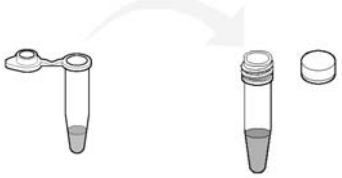
1 Label one 1.5 ml **fliptop** micro tube for each of five fish samples. Also label one **screw-cap** micro tube for each fish sample.

2. Add 250 µl of Bio-Rad Laemmli sample buffer to each labeled **fliptop** microtube.

3. Cut a piece of each fish muscle about 0.25 x 0.25 x 0.25 cm^3 ( ) and transfer each piece into a labeled **fliptop** micro test tube. Close the lids.

4. Flick the microtubes 15 times to agitate the tissue in the sample buffer.

5. Incubate for 5 minutes at room temperature.



6. Carefully transfer the buffer by pouring from **each fliptop** microtube into a labeled **screw-cap** microtube. Do not transfer the fish!

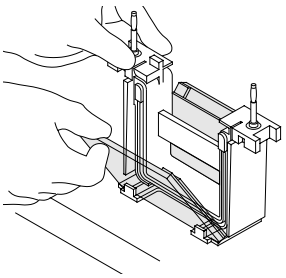
7. Heat the fish samples in screw-cap microtubes for 5 minutes at 95°C.

Lesson 2 Quick Guide

1. Set up Mini-PROTEAN 3 gel box.

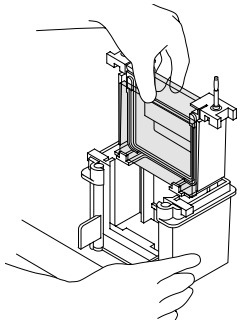
2. Prepare a Ready Gel cassette by cutting along the black line on the bottom of the cassette with a razor blade and pulling off the plastic strip, as indicated on gel cassette.

3. Remove the comb from the Ready Gel cassette.

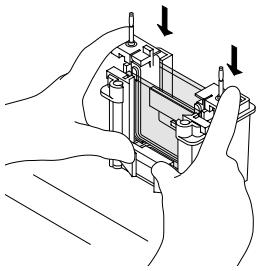


4. Place Ready Gel cassette into the electrode assembly with the short plate facing inward. Place a buffer dam or another Ready Gel cassette on the opposite side of the electrode assembly, with notch on buffer dam facing inward.

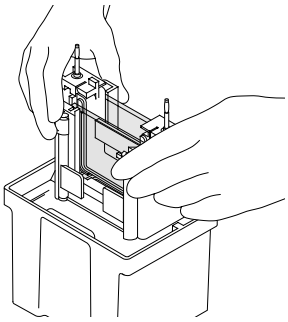
5. Slide gel cassette, buffer dam, and electrode assembly into the clamping frame.



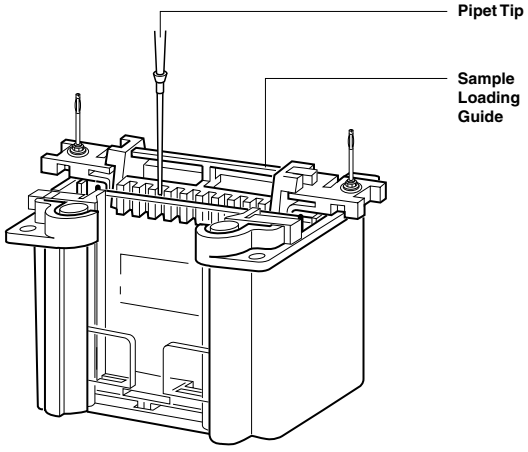
6. Press down the electrode assembly while closing the two cam levers of the clamping frame.



7. Lower the inner chamber into the mini tank.



8. Completely fill the inner chamber with 1x TGS gel running buffer, making sure the buffer covers the short plate (~150 ml).
9. Fill mini tank approximately 200 ml of 1x TGS gel running buffer.

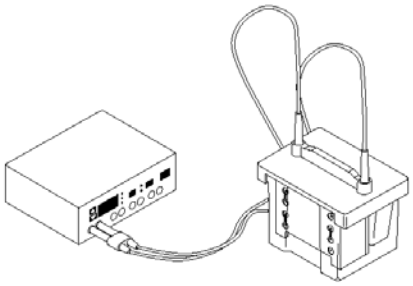


10. Plate sample loading guide on top of the electrode assembly.

11. Heat fish samples and actin and myosin standard to 95°C for 2–5 min.

12. Load your gel:

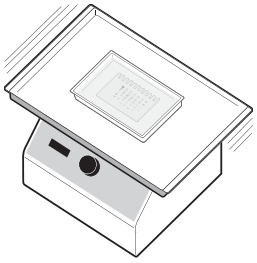
Lane	Volume	Sample
1 & 2	empty	Empty
3	5 µl	Precision Plus Protein Kaleidoscope prestained standards
4	5 µl	fish sample 1
5	5 µl	fish sample 2
6	5 µl	fish sample 3
7	5 µl	fish sample 4
8	5 µl	fish sample 5
9	5 µl	actin and mysin standard (AM)
10	empty	empty



13. Electrophorese for 30 minutes at 200 V in 1x TGS gel running buffer.

14. Proceed directly to Lesson 3, continue to step 15 of Lesson 2 to stain gels or store gels overnight at 4°C.

15. If the gels are to be stained, save 50 ml of 1x TGS gel running buffer.



16. Remove gel from cassette and transfer gel to a container with 25 ml Bio-Safe Coomassie stain/per gel and stain gel for 1 hour, with gentle shaking for best results.

17. Discard stain and destain gels in a large volume of water for at least 30 minutes to overnight, changing the water at least once. Blue-stained bands will be visible on a clear gel after destaining.

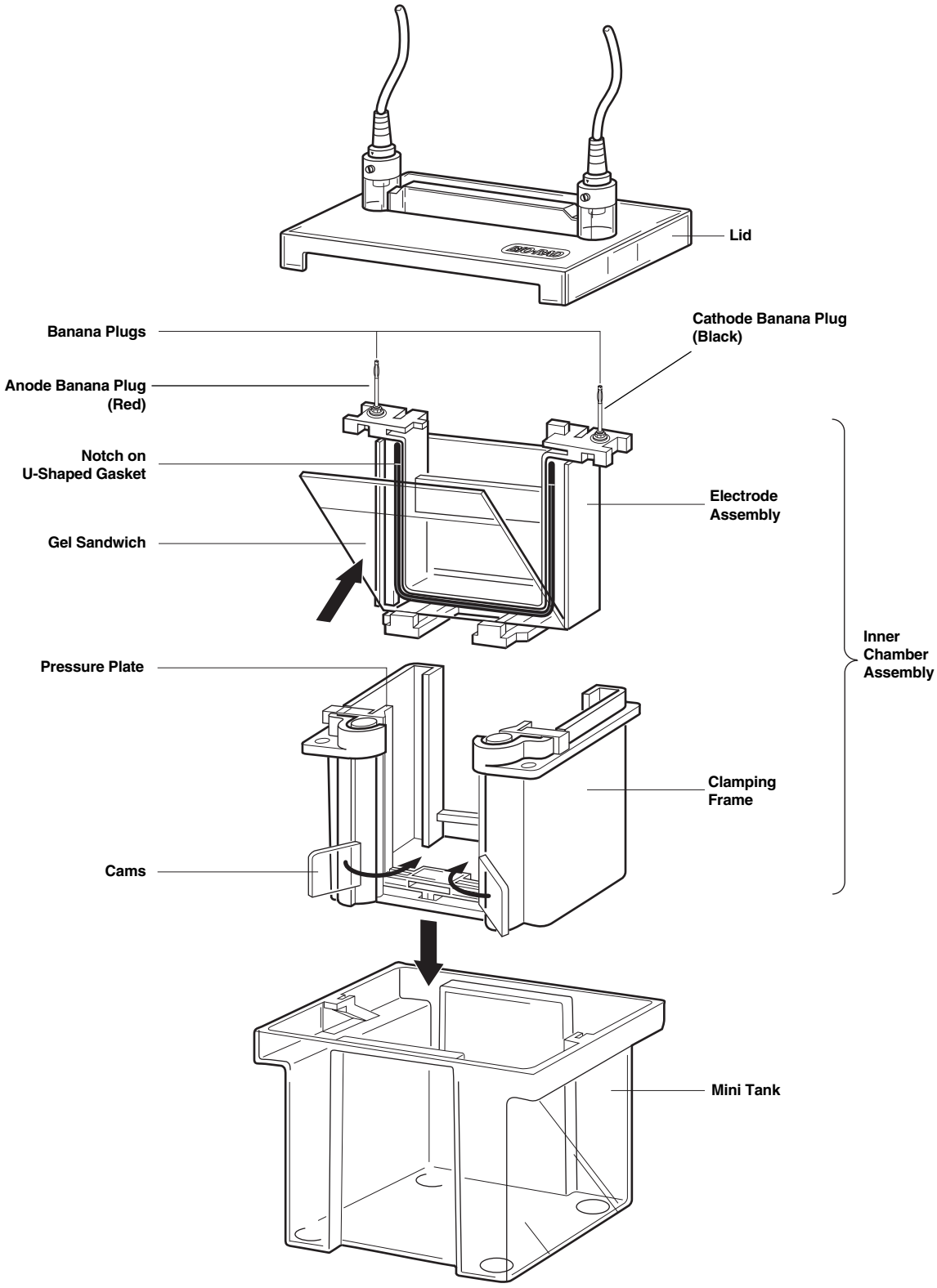
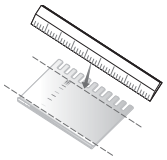
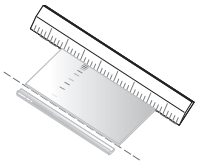
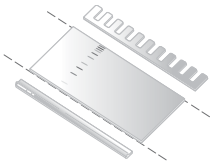


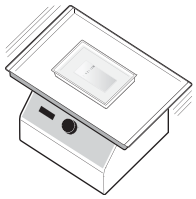
Fig. 4. Assembling the Mini-PROTEAN 3 cell.

Lesson 3 Quick Guide

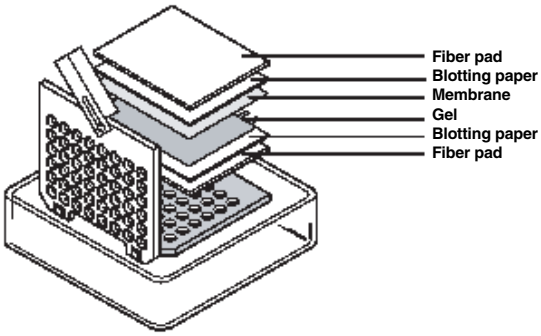
1. Using a ruler, chop the top and bottom off the gel.

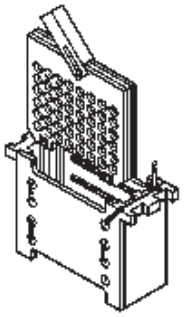
2. Equilibrate the gel in blotting buffer for 15 minutes on a rocking platform.



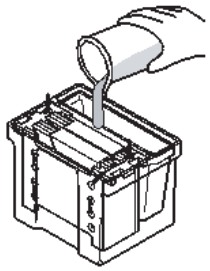
3. Soak fiber pads thoroughly in blotting buffer.
4. Mark the white nitrocellulose membrane with penciled (or black ball point pen) initials and prewet in blotting buffer along with the blotting paper.
5. Make the blotting sandwich:
   a. Add 1 cm depth of blotting buffer to container and insert plastic cassette with black side down.
   b. Lay a wet fiber pad on the black side of the cassette.
   c. Lay one wet blotting paper on the fiber pad and roll out air bubbles.
   d. Lay gel squarely on blotting paper and roll out air bubbles.
   e. Lay wet nitrocellulose membrane on the gel and roll out air bubbles.
   f. Lay one wet blotting paper on the membrane and roll out air bubbles.
   g. Lay a wet fiber pad on top of the blotting paper.



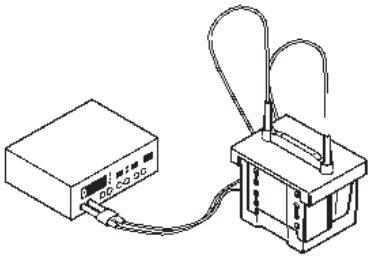
   h. Close the cassette and clamp together with the white clip.



6. Set up the Mini Trans-Blot module with the black side of the cassette next to the black side of the Mini Trans-Blot module. Add a frozen Bio-Ice module and fill with blotting buffer up to the white clip.



7. Place lid on tank, matching the power cords red-to-red and black-to-black, then blot at 20 V for 2.5 hours.



8. At this point the blots can be stored in the tanks submerged in blotting buffer at room temperature overnight or the sandwiches dismantled and the blots placed in blocker overnight at 4°C.

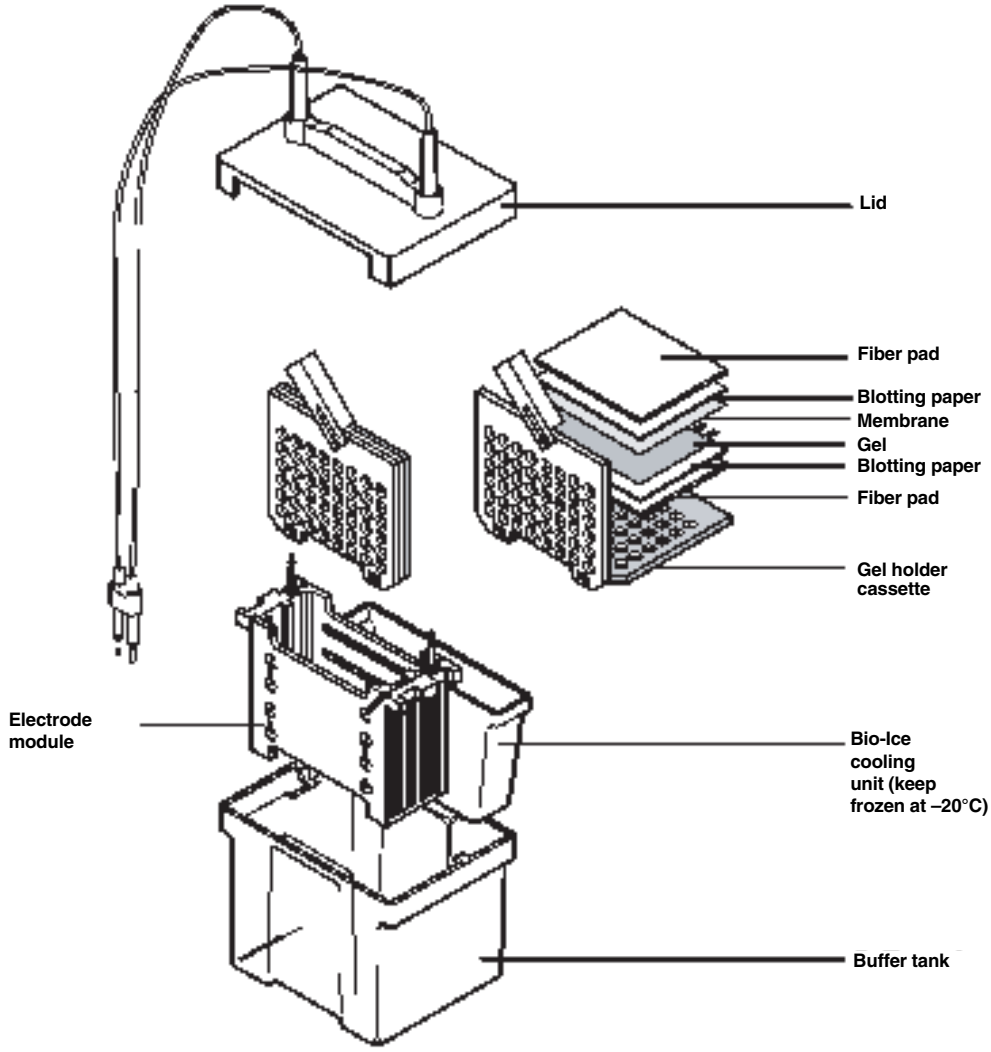
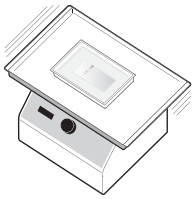


Fig. 5. Assembly of the Mini Trans-Blot cell.

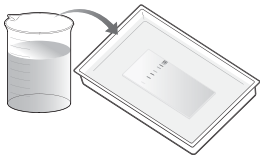
Lesson 4 Quick Guide

1. If not blocked overnight, immerse membrane in 25 ml blocking solution for 15 minutes to 2 hours at room temperature on a rocking platform.



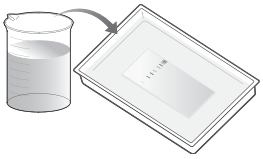
2. Discard blocking solution and incubate membrane with 10 ml of primary antibody for 10–20 minutes on rocking platform set to a faster setting to ensure constant coverage of the membrane.

3. Quickly rinse the membrane in 50 ml of wash buffer then discard the wash.
4. Add 50 ml of wash buffer to membrane for 3 minutes on rocking platform at a medium speed setting.



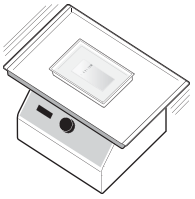
5. Discard the wash and incubate membrane with 10 ml of secondary antibody for 5–15 minutes on rocking platform set to a fast setting.

6. Quickly rinse the membrane in 50 ml of wash buffer and discard the wash.
7. Add 50 ml of wash buffer and wash membrane for 3 minutes on rocking platform on a medium speed setting.



8. Discard the wash and add 10 ml of HRP color detection reagent.

9. Incubate 10–30 minutes, either with manual shaking or on a rocking platform, and watch the color development.
10. Rinse the membrane twice with distilled water and blot dry with paper towel.
11. Air dry for 30 minutes to 1 hour and then cover in plastic wrap or tape in lab book.



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