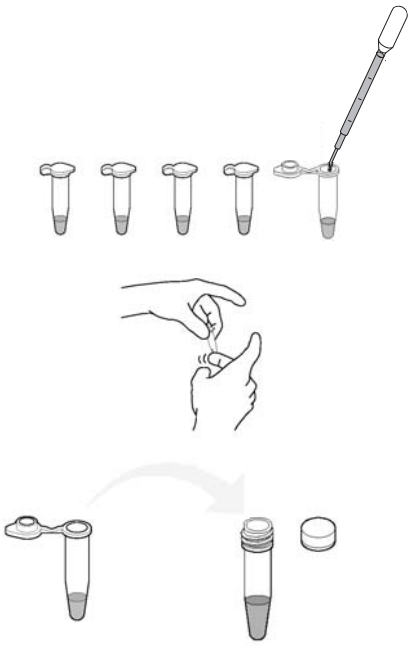
Comparative Proteomics Kit I: Protein Profiler Module – Quick Guide

Lesson 1 Quick Guide



1 Label one 1.5 ml **fliptop** micro tube for each of five fish samples. Also label one **screwcap** 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 **screwcap** microtube. Do not transfer the fish!

7. Heat the fish samples in screwcap microtubes for 5 minutes at 95°C.

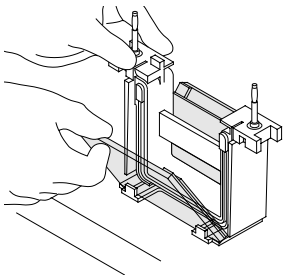
Lesson 2 Quick Guide

1. Set up Mini-PROTEAN 3 gel box and add 1x TGS electrophoresis buffer to the chamber.

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.

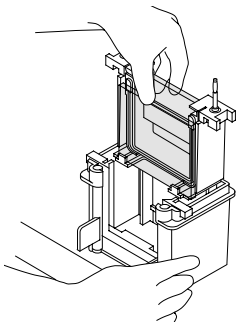
3. Remove the comb from the Ready Gel cassette.

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.

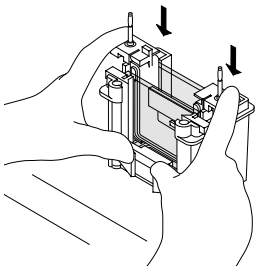


QUICK GUIDE

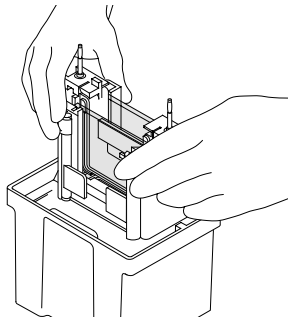
5. Slide gel cassette, buffer dam, and electrode assembly into the clamping frame.



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

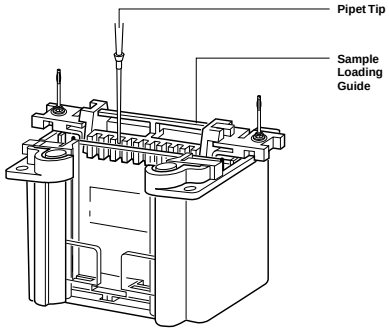


7. Lower the inner chamber into the mini tank.



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

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

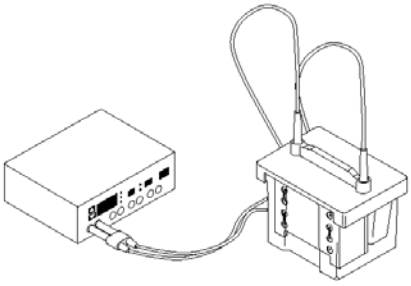


11. Heat fish samples and actin and myosin standard to 95°C for 2–5 min.

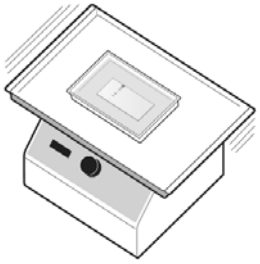
12. Load your gel:

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

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

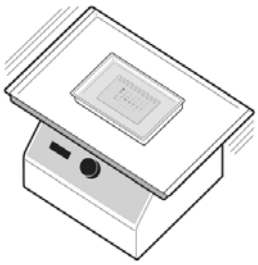


14. After electrophoresis, remove gel from cassette and transfer gel to a container with 25 ml Bio-Safe Coomassie blue stain per gel and stain gel for 1 hour, with gentle shaking for best results.



Lesson 3 Quick Guide

1. 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.
2. Dry gels using GelAir cellophane.



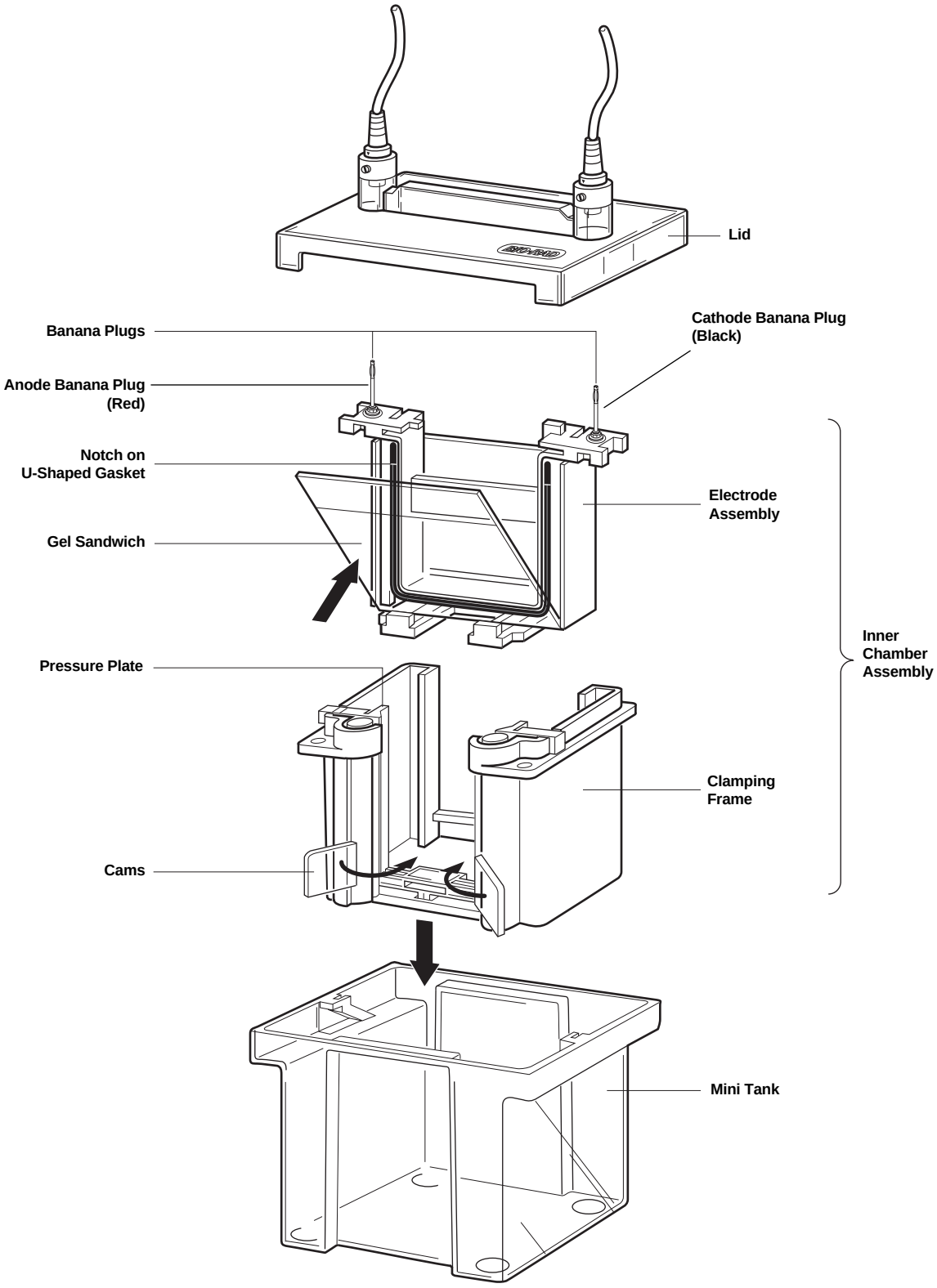


Fig. 11. Assembling the Mini-PROTEAN 3 cell.

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