

FluoWest™

Western Blot Procedure for Qdot® Conjugates

This procedure outlines the steps for western blot protein labeling from cell and tissue lysates. It assumes the user is familiar with cell lysates preparation and general polyacrylamide gel electrophoresis for subsequent western analysis.

Materials and Equipment

- Container for miniblots, 25 cm² container, or larger
- Electroblotting unit (Biorad or equivalent)
- Hybond ECL (from Amersham Biosciences, #RPN2020D, or other sizes) or PVDF low fluorescence membrane (from Amersham Biosciences, #RPN1416LFP). Important : PVDF membrane which is not low fluorescence can NOT be used.
- Power supply (capable of delivering 125V, constant voltage)
- Rocking or oscillating platform
- Primary antibody
- Qdot® secondary antibody conjugates
- 2 or more Sponges (approximately 11 cm²)
- Weigh boats
- Whatman #1 filter paper
- Gel imaging instrument (see below)

Buffers

Transfer buffer

100 ml 10X Tris-Glycine Buffer (Sigma, Cat# T-4904)

200 ml Methanol

Add distilled water to 1 Liter

Store at 4°C. Solution may be used twice before it must be replaced.

Blotting Buffers

Wash Buffer: 400 ml 10X TBS (Sigma Cat# T5912)
2 ml Tween-20
Distilled water to 4 liters.

Blocking Buffer: 100 ml 10X TBS
4% BSA, IgG-free (Sigma Cat # A9085)
0.05% sodium azide as preservative
Distilled water to 1 liter.

Antibody Buffer: 100 ml 10X TBS
4% BSA, IgG-free
0.05% Tween-20
Distilled water to 1 liter.

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE of protein lysates should be done as per routine. This procedure for blotting is for gels that are in the minigel format.

Note: Always wear gloves and use forceps when manipulating gels and membranes.

Gel to Blot Transfer – prepare blot membrane as per routine or use the procedure below.

1. Soak gel in Transfer Buffer for 10 minutes with gently rocking. If using a PVDF membrane, wet it in methanol for 1 minute, and rinse it with water for 5 minutes before soaking it in Transfer Buffer.
2. Cut 2 pieces of Whatman filter paper slightly larger than the size of the gel (at least as large as the membrane).
3. Gather together the sponges, the 2 pieces of Whatman paper, and the membrane and soak them in Transfer Buffer for 5 minutes prior to setting up the electroblotting apparatus.
4. Lay the electroblotting apparatus in a container that is at least 25 cm² containing approximately 250 ml cold Transfer Buffer.
5. Open the apparatus with the black side on the bottom of the container.
6. Assemble the transfer apparatus as follows, being careful not to introduce any air bubbles between the layers:

Negative electrode
Sponge
Filter paper
Acrylamide gel
Membrane
Filter paper
Sponge
Positive electrode

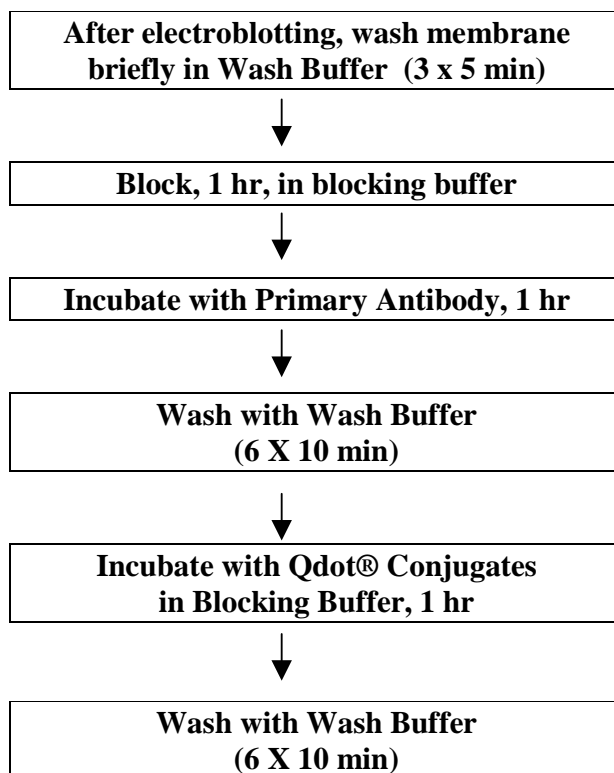
(if using PVDF membrane is used, place the smooth side against the gel)

7. Pour 1 liter of cold Transfer Buffer into the tank.
8. Close the sandwich apparatus making sure that the clasps are shut tight (more Whatman filter papers should be added if it is not tight), and place it into one of the slots of the tank (The black side faces the black side of the cassette). Connect the black negative electrode to the terminal nearest the black side of the sandwich apparatus. The red positive electrode should be placed closest to the membrane (transferred proteins have a net negative charge from the SDS).
9. Transfer the entire unit to 4°C and electroblot at 100V for 1-1.5 hours. Outline the gel with a dull pencil onto the membrane and carefully lift the gel to check for transfer of the pre-stained MW markers. The higher molecular weight standards are more difficult to completely transfer; it is alright if some of those markers remain on the gel. If transfer of the lower molecular weight standards is not yet complete, re-assemble the apparatus, carefully replacing the gel exactly as it had been on the membrane; return the unit to 4°C and resume electroblotting.
10. When molecular weight markers have transferred, remove membrane and proceed to step 11.

Blot Labeling

11. Wash membrane 3X in 10 ml wash Buffer for 5 minutes each with gentle shaking.
12. Place the membrane in 5 or 10 ml Blocking Buffer for at least 1 hour at 37°C or overnight at 4°C, rocking constantly.
13. Dilute the primary antibody to 1 µg/ml in 10 ml final volume of Antibody Buffer and add the membrane. (The amount of primary antibody may be optimized according to each specific experiment.)
14. Incubate the membrane at room temperature for 1 hour with constant rocking.
15. Wash membrane 6X in 10 ml Wash Buffer for 10 minutes each with gentle shaking.
16. **Dilute Qdot secondary conjugates to 1/1000e** in Antibody Buffer and add the membrane.

17. **Incubate the membrane at room temperature for 60 minutes** with constant rocking.
18. Wash membrane 6X in 20 ml Wash Buffer for 10 minutes each with gentle shaking.



Blot Image capture.

Place blot membrane onto clean glass surface and siphon excess liquid from edge of blot and position in a gel imaging system.

Western blots labeled with Qdot conjugates can be imaged in gel imaging systems equipped with a high quality digital camera and long wavelength UV epi-illumination, and band-pass filters matched to the quantum dot emission wavelengths. Trans-illumination may also be used but the autofluorescent light from the membrane is significantly higher. Follow your gel imaging manufacture procedure to grab the image.

For best results, we recommend the use of the G:Box Chemi HR or G:Box Chemi HR16 or G:Box Chemi XT 16 systems from SynGene (image capturing protocol included).