

Protein Transfer—Bio-Rad Criterion Blotter
AfCS Procedure Protocol PP000000500
Version 1, 01/17/02

The following procedure is the middle step of the 3-step process of Western immunoblotting. The first step is sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) to resolve a mixture of proteins by size (see AfCS Protocol SDS-PAGE—Bio-Rad Criterion Cell). The second step, detailed in this protocol, is the transfer of resolved proteins from the gel to a membrane support (i.e., nitrocellulose or polyvinylidene difluoride [PVDF]) via electroelution. The product of this second step is referred to as the blot. The third and final step is processing of the blot for detection of specific proteins with an antibody, the product of which is the final Western immunoblot (see AfCS *Protocol Western Blot Analysis—Phosphoprotein-Specific Antibody Cocktail*, PP000000700 or *Western Blot Analysis—Phosphotyrosine*, PP000000600).

Preparation

1. Run SDS-PAGE per AfCS Protocol SDS-PAGE—Bio-Rad Criterion Cell.
2. Prepare chilled (4 °C) protein transfer buffer—Bio-Rad (Bio-Rad transfer buffer). Cut the nitrocellulose or PVDF membrane (1 per gel) and filter paper (2 per gel) to the dimensions of the gel. Conduct the following steps in a cold room. If using nitrocellulose, soak it in purified water until needed. Alternatively, activate PVDF by submerging in methanol for 2 to 3 sec; place in purified water until needed. (Note: this should not be done for nitrocellulose, which will dissolve in methanol.)
3. Slowly submerge fiber pads (2 per gel) (starting with one end) into a container that will sufficiently allow the fiber pads to be immersed in 1 L of Bio-Rad transfer buffer until completely wet. Press down the pads while they are submerged to remove air bubbles trapped inside pads. Seal the container with plastic wrap and/or lid. Continue to soak fiber pads for at least 30 min.

Assembly of Gel/Blot Sandwich

4. Place the colored gel holder cassette in the back/large compartment of the gel blot assembly tray. Open the cassette so that the red side (anode) with the handle is vertical and the black side (cathode) is horizontal and submerged in the Bio-Rad transfer buffer.
5. Move fiber pad (presoaked in step 3) from preparation container to front compartment of tray. Remove bubbles with Bio-Rad roller. Place one fiber pad on top of the black side of the cassette submerged in the buffer.
6. Remove the gel cassette from the Criterion Cell after electrophoresis. Rinse gel cassette with chilled deionized water.
7. Use the cassette-opening tool built into the lid of the Criterion Cell to break the weld-joint on the gel cassette. Do this by turning the gel cassette upside down so that the upper buffer chamber fits over the built-in opening wedge of the lid; then firmly push the gel cassette straight down over the wedge on the lid until the weld-joint at the top of the cassette is broken and the two cassette halves can be pulled apart. The gel should remain adhered to the front plate of the cassette, leaving the back of the gel exposed (the front plate is the plate on which the wells are marked). This orientation is important for the final imaging of the blots. If the gel is not

adhered to the front plate, the orientation will be reversed. In this case, select the image transposition option before scanning the Western immunoblot.

8. Cut the bottom of the gel carefully with the gel knife. Rinse the gel by submerging it briefly in the chilled Bio-Rad transfer buffer (present in tray or preparation container). Place a piece of filter paper on the exposed backside of the gel and carefully separate the gel from the plate, keeping the gel adhered to the filter paper so that now the front side of the gel is exposed.
9. Place the filter paper with the gel on top of the fiber pad, with the front of the gel facing up. Use Bio-Rad roller to gently remove any bubbles that may be trapped underneath the gel.
10. Take the precut nitrocellulose or activated PVDF membrane presoaked in purified water and place it over the front side of the gel. Roll out bubbles.
11. Wet filter paper in Bio-Rad transfer buffer and place it on top of the membrane. Roll gently to remove any air bubbles trapped in the sandwich.
12. Move another fiber pad (presoaked in step 3) from preparation container to front compartment of tray. Use roller to remove air bubbles and place pad on top of the second filter paper.
13. Lower the red side of the gel holder cassette and lock the handle into the closed position.
14. If transferring a second gel, repeat *Assembly of Gel/Blot Sandwich* steps above.

Electroelution

15. Move the locked gel holder cassettes from the assembly tray into the groove of the Criterion Blotter tank, aligning the red side of the cassette with the red electrode.
16. Add the remaining Bio-Rad transfer buffer (from tray and preparation container) to the fill level marked on the tank.
17. Place the lid on top of the unit.
18. Connect the electrode cables to the power supply.
19. Turn on the power supply, usually at constant voltage of 100 volts for 40 min.

Disassembly of Gel/Blot Sandwich

20. Remove the gel holder cassette from tank and open. Remove all filter pads; then remove the filter paper that is directly attached to the membrane. Hold the rest of the filter paper/gel/blot sandwich and gently remove the blot from the gel.
21. Place the filter paper with adhered gel into a shallow dish with purified water and let sit for 5 to 10 min. Gently remove the filter paper, being careful not to tear the gel. Discard purified water from the dish and replace it with Coomassie Blue protein staining solution (to view extent of protein not transferred) or, under less frequent circumstances, dispose of gel without staining.
22. To store blot, keep it damp by wrapping in plastic with accompanying filter paper and storing at -20°C , unless blot will be processed immediately.
23. Place the blot in a container with Ponceau S stain solution for 15 min to evaluate the efficiency of protein transfer. Quickly rinse the blot with purified water 4 or 5 times. Place each blot in a transparent sheet protector and immediately scan or make a photocopy.

24. Wash blot for 5 min in Tris-buffered saline, pH 7.6, 1X (1X TBS) to prepare for Western immunoblotting (AfCS Protocol Western Blot Analysis—Phosphoprotein-Specific Antibody Cocktail or Western Blot Analysis—Phosphotyrosine).

Reagents and Materials

Protein transfer buffer—Bio-Rad (Bio-Rad transfer buffer), 1.5 L: AfCS Solution Protocol ID PS0000004800

Criterion Blotter: Bio-Rad; catalog no. 170-4070

Includes tank with plate electrodes; lid with power cables; gel holder cassettes; gel blot assembly tray; sealed ice cooler unit; fiber pads; and roller

Nitrocellulose membrane: Bio-Rad; catalog no. 162-0115

Immuno-Blot polyvinylidene difluoride (PVDF) membrane: Bio-Rad; catalog no. 162-0177

Filter paper: Bio-Rad; catalog no. 162-0118

Ponceau S stain solution (Ponceau S), 500 ml: AfCS Solution Protocol ID PS0000004500

Coomassie Blue protein staining solution (Coomassie Blue stain), 1 L: AfCS Solution Protocol ID PS0000002100

Transparent sheet protector: C-Line Products; catalog no. 00010

Tris-buffered saline, pH 7.6, 1X (1X TBS), 1 L: AfCS Solution Protocol ID PS0000006300

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