12% SDS-PAGE Western Blot

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Abstract
This is the protocol for making and running a 12% SDS-PAGE Western blot. Protocol starts from pouring gels all the way to reading the membrane.

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Guidelines

Not all Western blots are going to work out. Prepare for failures.

Before start

Run a BCA assay before starting a Western Blot
Make 10% AP fresh each time you pour gels. Mix 0.1 grams of AP with 100 microliters of waters.

Protocol

Pouring Gels

Step 1.
Obtain materials Western Blot Gel Pouring Apparatus: green casting stands, 4 large glass plates, 4 small glass plates, plastic Western Blot holder for the casting stands, and combs.

Pouring Gels

Step 2.
Spray the glass plates with 70% ethanol before use and dry.

Pouring Gels

Step 3.
Place small plate on top of the large plates. Slide these two glasses into the green casting stands, making sure that the glass plates are even. Close the casting stand and clip them in place on the plastic Western Blot gel holders.

Pouring Gels

Step 4.
Grab the following materials for 12% SDS-PAGE gels: Lower buffer (Tris 0.5M-pH 8.8), upper buffer (Tris 1.5M-pH 6.8), water, 30% Acrylamide-Bis 37.5:1, 10% SDS, 10% AP, and TEMED.

Pouring Gels

Step 5.

Make the resolving gel first. Follow the recipe below. I usually make 4 gels at a time.

SDS-PAGE gel recipes

# gels	11		4		2	
	12% lower	5% upper	12% lower	5% upper	12% lower	5% upper
H_2O	27.5 mL	23.65 mL	10 mL	8.6 mL	5 mL	4.3 mL
Acryl/Bis 37.5:1 30% solution	33 mL	6.875 mL	12 mL	2.5 mL	6 mL	1.25 mL
Lower buffer*	20.625 mL	-	7.5 mL	-	3.75 mL	-
Upper buffer**	-	10.31 mL	-	3.75 mL	-	1.875 mL
10% SDS	825 µL	412.5 µL	300 µL	150 µl	150 µL	75 µL
AP 10% (100mg/ml)	550 µL	275 µL	200 µL	100 µL	100 µL	50 µL
TEMED	50 µL	50 µL	20 µL	20 µL	10 µL	10 µL
TOTAL VOLUME	82.5 mL	41.25 mL	30 mL	15 mL	15 mL	7.5 mL

*Lower buffer: 1.5M Tris base pH8.8

Weigh 90.85g of Tris base, dissolve in 350ml H_2O.

Adjust to pH8.8 with concentrated HCl.

Make up to 500ml with H_2O.

**Upper buffer: 0.5M Tris pH6.8

Weigh 30.25g of Tris base, dissolve in 350ml H_2O.

Adjust to pH6.8 with concentrated HCl.

Make up to 500ml with H_2O.

Pouring Gels

Step 6.

After making the resolving gel, use a 1000 microliter pipette to distribute the solution from the tube into the space between the glass plates. Stop pipetting the solution when you reach the bottom of the green section of the casting stand behind the glass plates. After pouring the gels, pipette some isopropanol on the top of the resolving gel to prevent evaporation. Let it polymerize for about 30 minutes or so. Use leftover solution from tube as a check to whether the gel is done polymerizing.

Pouring Gels

Step 7.

While the resolving gel is polymerizing, make the stacking gel but don't add the TEMED until you're ready to pour the stacking gel in. Follow the recipe below for the stacking gel.

SDS-PAGE gel recipes

# gels	11		4		2	
	12% lower	5% upper	12% lower	5% upper	12% lower	5% upper
H_2O	27.5 mL	23.65 mL	10 mL	8.6 mL	5 mL	4.3 mL
Acryl/Bis 37.5:1 30% solution	33 mL	6.875 mL	12 mL	2.5 mL	6 mL	1.25 mL
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Make up to 500ml with H_2O.

**Upper buffer: 0.5M Tris pH6.8

Weigh 30.25g of Tris base, dissolve in 350ml H_2O.

Adjust to pH6.8 with concentrated HCl.

Make up to 500ml with H_2O.

Pouring Gels

Step 8.

Once the resolving gel is done polymerizing, dump out the isopropanol in the sink and rinse with water.

Pouring Gels

Step 9.

Add the TEMED to your stacking gel solution and distribute the solution with the pipette until you reach the top of the short glass plate. Add the comb of your choice and make sure there is no bubbles. Let it polymerize for about 30 minutes. Use your tube of left over solution as a check.

Pouring Gels

Step 10.

After the gel is done polymerizing, you can store gels by wrapping them with moist paper towel and placing them in 4 degrees Celsius fridge until you're ready to use them.

Running the Gel

Step 11.

Before running the gel, make sure your samples have been denatured with sample buffer. If not, add the sample buffer to your samples and boil them for 5 minutes.

Running the Gel

Step 12.

Grab materials for running a gel: Western blot running container, lid with electrodes, 1X tank buffer, gels, samples, buffer dam (if running one gel), and gel holders.

Running the Gel

Step 13.

Place gels inside the gel holders and have the short plate and wells facing the interior of the gel holder.If you are only running one gel, use a buffer dam on the other side of your gels. Close them in place. Place the gels inside the tank.

Running the Gel

Step 14.

Fill the tank with 1X tank buffer until it reaches the appropriate line for how many gels you are running. Fill the interior of the gel holder with tank buffer to the very top.

Running the Gel

Step 15.

Load 10 microliters of the ladder of your choice into the first well. Transfer all your samples from the Eppendorf tubes into the wells of your gel.

Running the Gel

Step 16.

Place the lid on top of your Western blot apparatus with the electrodes matching with the corresponding color. Plug cables into the corresponding colors on the Western blot machines.

Running the Gel

Step 17.

Run the gel for 1 hour at 160V. Check on your gel every 8-10 minutes to make sure your gel isn't leaking with the tank buffer. Pause the machine and use a pipette to move buffer from the tank into the gel holder if it's leaking.

Transferring the Gel

Step 18.

Grab the following materials to make the transfer 'sandwich': transfer tank and lid, small magnetic stir bar, ice pack, transfer electrodegel cassette, 2 sponges, Whatman blot paper, Immobilon membrane, 1X transfer buffer, methanol, one plastic tub to fit membrane, and another tub to fill with transfer buffer.

Transferring the Gel

Step 19.

Fill a small plastic tub with methanol and pre-wet the Immobilon membrane with it to activate it.

Transferring the Gel

Step 20.

Dislodge your gel from the glass plates after the run. Use a razor blase to cut away the stacking gel.

Transferring the Gel

Step 21.

Assemble your sandwich as follows: Black side of the cassette, sponge, Whatman blotting paper, gel, Immobilon membrane, blotting paper, sponge, clear side of the cassette. Use the transfer buffer to keep the sandwich wet.

Transferring the Gel

Step 22.

Roll over your sandwich with a Sharpie marker to get rid of any bubbles.

Transferring the Gel

Step 23.

Close the sandwich and insert it into the transfer electrode with the black side of the cassette matching the black side of the electrode.

Transferring the Gel

Step 24.

Insert the transfer electrode into the transfer tank. Put the magnetic stir bar on the bottom of the tank and place the ice pack inside as well.

Transferring the Gel

Step 25.

Fill the tank with 1X transfer buffer.

Transferring the Gel

Step 26.

Put on the lid with the electrode matching the colors on the lid and plug the cables into the machine. Let the gel transfer for 2 hours at 0.4 A and let the stir bar spin at 50 rpm.

Transferring the Gel

Step 27.

The methanol and transfer buffer doesn't go down the sink and so can be re-used. Pour these back into their original containers.

Blocking the Membrane

Step 28.

After the transfer is done, place your membrane in a clear plastic container and put about 5 mL of blocking buffer in the container. Put this on a rocker and let it block for 1 hour.

Blocking the Membrane

Step 29.

While your membrane is blocking, thaw your primary antibodies.

Primary Antibodies

Step 30.

After blocking is done, dump the blocking buffer out. Pour the primary antibodies in the container and let it rock overnight in the cold room.

Primary Antibodies

Step 31.

After letting the primary antibodies rock overnight, pour it back into the original container. Grab your secondary antibodies and let it thaw.

Washing the Membrane Post-Primary

Step 32.

Wash your membrane three times with 1X PBS-Tween.

Secondary Antibodies

Step 33.

After washing, pour on your secondary antibodies after it has completely thawed. Secondary antibodies are light sensitive so wrap your container with aluminum foil.

Secondary Antibodies

Step 34.

Place your container on a rocker and let it rock for 1 hour.

Washing the Membrane Post-secondary

Step 35.

After 1 hour, wash the membrane three times with 1X PBS-Tween. Keep your container wrapped in foil while washing.

Washing the Membrane Post-secondary

Step 36.

After washing the membrane, take it out of the container and dry the membrane with 2 Whatman blotting

papers before analyzing it with the LI-COR machines.

LI-COR

Step 37.

Use the LI-COR machines to analyze your membrane.