Western Blot

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Abstract

How the Ju Lab does Western Blots

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Before start

Make sure you have a frozen ice block for the Bio-Rad container and have at least 800 mL of 1x Transfer Buffer chilled to 4 C.

Protocol

Step 1.

Place gels in the Bio-Rad gel running apparatus and make sure they are locked into place, then pour 1x Protein Running Buffer inbetween the two gels until it's about 25% full to ensure there are no leaks. After no leaks have been found, fill the middle up the rest of the way up so it covers the top of the wells and pour 1x Protein Running Buffer outside of the gels so it covers the bottom of the glass.

PROTOCOL

. 1x Protein Running Buffer

CONTACT: Alan Cone
Step 1.1.

25 mM Tris

Step 1.2.

190 mM glycine

Step 1.3.

0.1% SDS

Step 1.4.

20% Methanol

Step 1.5.

Check pH and adjust to 8.3 if necessary

Step 2.

Remove the combs from each gel and wash lanes a few times with 1x Protein Running Buffer, then load your samples and ladder. In any unused wells, fill them up with a similar volume that you loaded your samples with of 1x Protein Running Buffer.

Step 3.

Run 10% gels at 150 volts until the ladder reaches the bottom of the glass.

DURATION
01:30:00

Step 4.

Pour out 1x Protein Running Buffer (can reuse 1 time) into a bottle and then remove the gels from the gel apparatus. Separate the glass pieces, remove the gel, cut off and discard the stacking portion, and submerge the separating portion in 1x Transfer Buffer for at least 15 minutes

Step 5.

Cut nitrocellulose membrane and pieces of filter paper to the same size as the gel, and cut one corner so you are able to orient yourself to which side is the front or back, then soak in 100% methanol, followed by quickly submerging in 1x Transfer Buffer for at least 15 minutes.

NOTES
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Make sure you never touch the membrane with your bare hand, otherwise it'll leave a mark when you try to develop it.

Step 6.

Pour 1x Transfer Buffer into Bio-Rad transfer apparatus and soak both sponges and filter paper in the buffer.

Step 7.

Open up plastic sandwich, and starting with the clear side:

1. Place a wet sponge down
2. Place a wet piece of filter paper
3. Place the nitrocellulose membrane onto the filter paper (down in the middle first then let each

side down so you have no air bubbles)
4. Place the gel on the same way
5. Place the other piece of filter paper down the same way

Step 8.

Use a 15 mL centrifuge tube and roll from one side of the sandwich to the other to ensure there are no air bubbles.

Step 9.

Place the other sponge on top, then fold the black side up and lock the clear and black sides together so there is a complete sandwich, and load it into the center of the transfer apparatus, so that the black side of the sandwich matches the black side of the sandwich holder, and the clear side is closer to the red side of the sandwich holder.

Step 10.

Load the ice block into the apparatus then fill the Bio-Rad container to the top with chilled 1x Transfer Buffer.

Step 11.

Add the lid to the container, plug it into the amplifier, and perform the transfer at room temperature at 50 volts for two hours.

DURATION
02:00:00

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DO NOT INTERRUPT THE TRANSFER PROCESS OR YOUR SAMPLE WILL BE LOST.

Step 12.

Remove membrane from sandwich, rinse with water, and submerge in TBST + 5% Milk with shaking for two hours to initiate blocking.

DURATION
02:00:00

PROTOCOL

. TBST and 5% Milk

CONTACT: Alan Cone
Step 12.1.

20 mM Tris pH 7.5

Step 12.2.

150 mM NaCl

Step 12.3.

0.1% Tween 2

Step 12.4.

5% Dry Milk

Step 13.

Dump the TBST + Milk, then add 10 mL of fresh TBST + 5% Milk and 1 uL Primary Antibody (all mixed together before submerging the membrane in it) and let it rock at 4 C from two hours to overnight.

DURATION
02:00:00

PROTOCOL

. TBST and 5% Milk

CONTACT: Alan Cone

NOTES
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The specific amount of your antibody to add will be based on antibody specifics, for our lab and our set of antibodies this ratio works well.

Step 13.1.

20 mM Tris pH 7.5

Step 13.2.

150 mM NaCl

Step 13.3.

0.1% Tween 2

Step 13.4.

5% Dry Milk

Step 14.

Pour off TBST and 5% Milk with the Antibody and do five 10 minute washes with TBST and shaking at room temperature.

DURATION
00:50:00

PROTOCOL

. 1x TBST

CONTACT: Alan Cone
Step 14.1.

20 mM Tris pH 7.5

Step 14.2.

150 mM NaCl

Step 14.3.

0.1% Tween 20

Step 15.

Dump the TBST, then add 10 mL of fresh TBST + 5% Milk and 1 uL Secondary Antibody (all mixed together before submerging the membrane in it) and let it incubate while shaking at room temperature for 1 hour.

DURATION
01:00:00
Step 16.

Pour off TBST and 5% Milk with the Antibody and do five 10 minute washes with TBST and shaking at room temperature.

DURATION
00:50:00
Step 17.

Add just enough BCIP/NBT to cover the membrane and let it shake at room temperature until bands develop.

NOTES
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If the membrane starts turning purple then it has been exposed for too long.