

5X Lamelli Buffer

0.5M Tris-HCL pH6.8	1.75ml
Glycerol(Glycerin)	4.5ml
SDS (0.25g dissolved in 1ml Thris-HCL)	2ml 0.5g total
0.25% Bromophenol blue (25mg in 10ml H2O)	0.5ml
B-mercaptoethanol	1.25ml
	Total of 10mls

Store in fridge and protect from light

1x Lamelli buffer

Bio Rad Lamelli sample buffer

950ul sample buffer and 50ul BMe

Stock Buffer (Immunoblots)—10 L

144 g glycine

30.5 g Tris Base

10 L dH2O

Running—1L

1 g SDS

1 L stock buffer

Transfer, PVDF—1 L

1 L stock buffer

--PVDF membranes are washed in methanol before use

Transfer, nitrocellulose—1 L

800 mL stock buffer

200 mL methanol

Basic Westerns

Harvest cells

Remove media
Wash 2x with 1x PBS
Scrape cells into 600ul 1xPBS
Place cells and PBS into ice cold 1.7ml tubes-labeled
Use the ice cold glass pestle to break up the cell clumps ~2mins
Spin 13,000rpm for 10-20mins
Remove the supernatant to a new labeled tube
Remove 100ul of the supernatant for protein analysis
After you have analyzed the amount of protein take 40ug of protein that's in the Supernatant, add 2x volume of Lamelli buffer to your 40ug to load gel.

Loading gel

Make sure you have enough "running buffer" if not make some up.
Make sure your protein sample has 2x Lamelli buffer added to it
Heat 95-100 for 5 mins
Set up your gel rig and figure the orientation for your samples and marker
Load 10ul marker and appropriate amount of sample to wells
Run at 200v for an hour or until the dye runs off, on ice or 20v overnight in the cold room

Transfer

Make sure you have transfer buffer made up
Trim your membrane to fit your gel
If using PVDF make sure you soak the membrane in Methanol until translucent then wash with DI water 2x
Set up your transfer "book" Black on the bottom, then sponges, then filter paper and finally gel. Place membrane on top of gel and roll out air bubbles before placing the filter paper on top then the sponge then the clear side of the book. Slide to lock in place.
Place in transfer rig black to black and clear to red
Place lid on black to black and red to red
Run transfer at 100v for an hour
Carefully check to make sure marker transferred before completely taking apart "book". If needed, close book and transfer for longer

Ponceau

Once you are done transferring place transferred blot into tub marked for ponceau and pour ponceau solution directly onto blot and allow to sit and mix for 5 mins.

Rinse with TBST and look for bands on blot where your sample was added. Once you see the bands wash completely with TBST

Block

Use 5% milk, 2.5g to a 50ml conical and fill with TBST

Allow time to mix well

Once milk is dissolved place blot in a container with milk and allow to rock for an Hour

Primary AB

Once you are done blocking add your primary antibody 1:100 and 1:1000, two different containers.

Cover and place in cold room overnight

Secondary AB

Take your blot out of the cold room and wash with TBST 2x for 10min each

Add 5% milk to blot and then your secondary AB, either mouse or rabbit, at

1:1000 and 1:5000 respectfully to your primary dilution

Cover and rock for at least an hour

Exposing

Wash your blot that's been in secondary 3x with TBST at 10 mins each

Take separate container and add 1ml of each Supersignal reagent.

Add your blots and pipette the reagents over then blots for 2 mins

Place blots on Kim wipes and dry by folding Kim wipes over the blot

Place dried blots into film cassette

Go to darkroom and place film on covered blots for 2mins, turn/flip film place on top of blots for 5 mins. Depending on how many blots you have at same

time use two pieces of film for 2,5,10 and 1 min exposures.

Place film into developer and allow film to come out on outside.

General Licor Odyssey Protocol

Loading gel

1. Make sure you have enough “*running buffer*” if not make some up.
2. Make sure your protein sample has *Lamelli buffer* added to it
3. Heat samples 95-100C for 1-5 mins
4. Set up your gel rig and figure the orientation for your samples and mol weight marker
5. Load 2-7ul of mol. weight marker and appropriate amount of sample to wells. 45ul for 12+2 criterion or 50ul for 10 wells in a ready gel
6. Run at 200v for an hour or until the dye runs off the gel

Transfer

1. To Nitrocellulose, 0.2um, using *Nitrocellulose transfer buffer*
2. Run 200-150V for an hour or 20V Overnight in cold room
3. Place blot into 1xPBS to rinse off the methanol

Block

1. Move blot to Licor blocking solution and 1xPBS 1:1 NO TWEEN for an hour

Primary

1. To the block solution add primary AB and 0.05% tween 20
2. Place blot in cold room on rocker O/N

Wash

1. 4x with 1xPBS and 0.05% tween 10mins/wash

Secondary

1. Licor blocking solution, 1xPBS 1:1, and 0.05% tween 20
2. Licor secondary AB, 680-800. We use a 1:15,000 concentration
The 800 is for your more sensitive probe, 680-700 is for more standard probes
3. Place on rocker for an hour at RT

Wash

1. 4x with 1xPBS 0.05% tween20 10mins/wash
2. 2x with JUST 1xPBS 10min/wash

Image blot using the Licor Odyssey