SDS-PAGE Running Buffer (for TRIS/Glycine gel)

Trizma Base	25mM (MW 121.1)	pH 8.3	3.02g/L
Glycine	192mM (MW 75.07)		14.41g/L
Sodium Dodecyl Sulfate	0.1% (MW 288.38)		10mL/L (10% stock)
H2O			

Make 1L 10X Running Buffer

30.29g	Tris Base
144.10g	Glycine
100mL	SDS (10% stock) or 10g/L
--1L Total	

Add all chemicals to water. Stir till dissolved. Adjust pH to 8.3

Transfer Buffer

25mM Tris base	3.03g/L
192mM glycine	14.41g/L
20% methanol	

Make 1L 10X transfer buffer

30.29g	Tris Base
144.10g	Glycine
QS	H2O
--1L Total	

Add Methanol fresh each time

1L Transfer Buffer

100mL	(10X) Transfer Buffer
200mL	Methanol (20% final)
700mL	H2O
--1L Total	

Western Blotting

Stain the membrane with ponceau solution (>3 min)
wash the membrane with H2O several times (5min x 3)
Block the membrane with 5% milk/PBST
Wash membrane with PBST three times (10minx3)
Add 1x Primary antibody and incubate 1 hour on the rotator
Wash membrane with PBST (10minx3)
Add 1x Secondary antibody and incubate for >1hour on the rotator
Wash membrane with PBST (10minx3)

Remove all liquid and add 1ml ECL
Use Fuji imager in 300NE

Fuji Imager reservation:
http://www.brownbearsw.com/cal/fuji301

Username: Fuji 301
Password: Fuji301

Reagents

PBST
    1xPBS with 0.2% Tween

Blocking solution
    5% milk in PBST

Primary antibody
    antibody in 3% BSA/PBST (re-use it for few months)

Secondary antibody
    Secondary antibody in 5% milk/PBST (discard it each time after use)