WESTERN BLOTTING

Transfer Buffer: for 1L
5.8 g Tris Base
2.9 g glycine
0.37 g SDS
---Make to 800 mL with dH_2O, then add 200 mL MeOH---

Blocking Solution: for 1L
10 g powdered nonfat milk (1%)
500 uL Tween 20 (0.05%)
Make to 1L with 1X PBS
Store at 4°C for no more than 1 week.

Horseradish Peroxidase Developer:
10 mL MeOH
30 mg 4-chloro-1-naphthol
Make to 50 mL with 1X PBS.
Add 10 uL H_2O_2 and mix.
Good for less than 1 hour.

Alkaline Phosphatase Developer:
APC Solution
100 mM Tris-HCl
100 mM NaCl
5 mM $MgCl_2$
pH to 9.5
For 20 mL APC Solution, add 66 uL 5-bromo-4-chloro-3-indolyl phosphate and 132 uL of nitroblue tetrazolamine.

Run gel at 10-15 mAmps/gel.
Transfer either at 350 mAmps at RT or 100 mAmps overnight.
To set up:
Soak everything in Transfer buffer, including gel.
Lay blotting pad on black side of transfer case.
Next lay filter paper, gel, and membrane.
Make sure there are no bubbles between gel and filter paper or gel and membrane.
On membrane place filter paper and then blotting pad and close the transfer case.
Place into gel box with the black side facing the black side of the transfer case holder.
Place ice block in gel box and fill with transfer buffer.

Once transfer is done, place membrane in blocking solution for 1 hour at room temperature.
Then add 15 mL blocking solution + antibody at proper dilution (anti-GFP 1:2000, Pats1 antibodies 1:1000, Hsc70-2 antibody 1:5000).
Place on shaker for 3 hours at RT or overnight at 4ºC.
Wash 3X with blocking solution.
Add 15 mL blocking solution + antibody. (1:1000 for goat anti-rabbit HRP conjugate, and 1:2000 for Anti-mouse alkaline phosphatase conjugate).
Place on shaker for 1 hour at RT.
Wash 2X with 1XPBS+0.05% Tween20.
Wash 1X with 1XPBS.
Develop using appropriate developer and stop for 1 min in water.