iGEM 2019 Western Blot Protocol

Gel Electrophoresis

1. Prepare running buffer with 10X Tris/Glycine/SDS. The recipe for this can be found below. You can make 1L of this and use it for future western blots.
2. Heat samples at 95oC, for 10 minutes.
3. Spin briefly (14K rpm, ~1 min)
4. Meanwhile heating and spinning samples:
    a. Remove gel from storage pouch, rinse briefly with water. (4-20% gradient gel)
    b. Remove the green tape from the bottom of the gel.
    c. Assemble the electrophoresis module according to manual.
    d. Fill the inner chamber with buffer, to just under the edge of the outer gel plate. Make sure it is not leaking…
    e. If it is leaking, fill the outer chamber to the 4-gels mark. If it is not leaking and you have only 2 gels, you can fill the outer chamber to the 2 gels mark.
    f. Rinse the wells with the buffer in the chamber, using gel loading tips.

5. Load the samples into the gel (20-100 ug protein/well). Load the ladder in one of the middle wells.
6. Run at 120 V, ~2 min, until you can see that the blue dye is inside the gel.
7. Run at 185 V for 30 min. Run extra couple of minutes if you want to separate the smaller peptides more.
8. Rinse electrophoresis setup with distilled water.
9. (Optional: Activate the gels if they need it).
10. Image gels (Use the Stain Free Tray, Image Settings: Protein: Stain Free Gel)

Protein Transfer

1. Use the Transfer Pack and assemble the transfer cassette according to the BioRad Transblot Turbo manual.
2. Make sure to eliminate bubbles before running the protein transfer protocol.
3. Plase the casettes in the machine and turn it on. Select Turbo and MINI as the gel format. Press Run. (The run will take 7 minutes)
4. If dry spots appear on the blot; rinse it briefly in EtOH.
5. Keep the blot in TBST before imaging, to keep it from getting dry.
6. Image blots. (Stain free blot/ Protein blot)

Antibody Probing

1. Incubate the blot with Western Blocker Solution for 1 hour or overnight in 4 ^{o}C.
2. Incubate 1 hour with primary AB. (Diluted 1:1000 in Western Blocker)
3. Make sure not to throw away the AB. It can be reused.
4. Wash with TBST: 5 min X 4 times.
5. Incubate 1 hour with secondary AB (Diluted 1:1000 in MQ water)
6. Wash with TBST: 5 min X 6 times.
7. Prepare HRP: Mix 1:1.

8. Incubate the blot with HRP for about 5 minutes, RT. Light sensitive.
9. Image immediately. (Multichannel: Colorimetric, Hi Senitivity)