BN-GEL protocol (Cyanobacteria thylakoid)

1. Thylakoid preparation follows lab routine (50ml or 200 ml culture). Pay attention of the soft pellet when dumping the supernatant after centrifugation. PSII prep differently
2. Resuspend in BNRB at 1mg/ml of [Chl].

BNRB: Blue Native Resuspend Buffer (50 mM Bis-tris, 750 m 6-amino-caproic acid (ACA), 0.5 EDTA, protease inhibitor cocktail (add before use)).

1. Membrane is solubilized by adding one volume of freshly made solubilization buffer. (4% dodecyl maltoside dissolved in BNRB). Add 1/5 of total solubilization buffer each time and mix well until finish all of the solubilization buffer. Incubate at 4°C on a shaker for 1 hour (or longer, Feb 5th result is from Feb 4th solubilized sample supernatant).
2. Spin at 12,500 rpm (SS-34) for 30 min. Supernatant [Chl] is about 0.8 mg/ml. Add 1/20 volume of BNSB (Blue Native Sample Buffer, 5% G250, 100 mM Bis-Tris HCl, 500 mM ACA, 30% sucrose). Mix
3. Loading 8ul (about 8x0.8=6.4ug chl) to 1 mm thick gel (comb width 10 mm) or 8ul 1.5 mm thick Hoefer gel (comb width). For 0.75 mm gel, loading volume = ul.
4. For two gels, one Hoefer (1.5 mm) and one Atta 1.0 mm gel. 18 mA constant current was used for the first 1 hour 36 min and followed by 16 mA constant current for 1 hour 6 min.

Gel preparation:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Stacking gel 4% | Separation 5% | Separation 13.5% | Separation 16% |
| 49.5% Acr (ml) | 0.242 | 0.212 | 0.573 | 0.68 |
| 3X Gel buffer | 1.0 | 0.7 | 0.7 | 0.7 |
| H2O | 1.72 | 1.028 | 0.247 | 0.14 |
| 75% Glycerol | 0 | 0.14 | 0.56 | 0.56 |
| TEMED | 6ul |  |  |  |
| 10% APS | 32ul |  |  |  |
| Total (ml) | 3.0 | 2.1 | 2.1 | 2.1 |

1. Fill the tubing with H2O (It’ll be on the top of the gel for sealing the gradient gel) and connect to gradient maker and long needle.
2. Hoefer 1.5 mm gel, first, pour 17ml to 5% column of gradient maker and open the switch between two columns and let some solution fill the connection tubing, remove any 5% gel solution from 13.5% column to 5% column. Add 17ml 13.5% gel solution to the column. Open the switch between two columns, start stir plate, add 20 ul and 10 ul TEMED to 5% and 13.5% gel respectively, and 110 and 50 ul APS (10%) to 5% and 13.5% gel respectively. (For 1 mm gel, use 10.5 ml to each column of the gradient maker. For 0.75 mm gel, use 8.4 ml to each column).
3. Leave small amount of H2O in the tubing connecting the gradient maker and the long needle (dripping 40 drops water from full tubing or more for the 0.75 mm Hoefer gel). Put long needle in between the two plates and let the tip touch the bottom of the gel caster. Use maximum pump speed (0.2 ml/sec) to fill the space between the plates. Let the sealing water approach the top of the plates. Stop and clean. Let the gel stand 45min for polymerization, due to the low percentage of the gel, it’s not easy to see the polymerization line between sealing water and 5% gel.
4. Prepare plenty of sample gel and wash the polymerized gel twice with it. Assemble the comb in place, set the distance between comb and separation gel of 4-5 mm (can be less than that but no more).
5. Remove the comb carefully when polymerization is ready. Load sample with Hamilton syringe (8ul). Fill the well with cathode buffer to the top. Assemble the gel cassette and set 11 mA constant current for 1.5 mm gel (8 mA for 1.0 mm gel, 6 mA for 0.75 mm gel).
6. Change cathode buffer without CBB G-250 after 1.5 hour. And run another 1 hour to remove G- 250.

Blotting:

1. Soak the gel in transfer buffer (ss+SDS) and assemble PVDF membrane25 Voltage 3h at 4C.

From 62509 protocol: (0.75 mm gel Hoefer)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Chl ug (ul)\* | BNRB ul | BNRB (2%DM+PIC) | BNSB ul | Loading |
| HT47PSII | 5 (2.4ul) | 7.6 ul | 10 ul | 1 |  |
| pHisF | 2 (10ul) | 0 | 10 ul | 1 |  |

Loading 1 ug chl or 0.75 ug (3ul)

How about PSII sample in RB? Combine His-column fraction in a 1:1 ratio to BNRB (2%DM+PIC) and add 1/20 volume of BNSB.

Marker protein (Invitrogen)