V-GES operation

MATERIAL

- BSA (1 mg/ml), 0.05 g BSA (Sigma-Aldrich Ltd., St Louis, MO; U.S.A.) dissolved in 50 ml ddH_2O, aliquot 1 ml solution per Eppendorf and freeze in -20℃.
- Pre-stained marker (Bio-Rad, Hercules, CA, U.S.A.)
- ProMarker (Wealtec, Taipei, Taiwan)
- Two sets of glass plates with alignment card, thick glass plate and U-shaped glass plate, 0.75 mm spacers and one 0.75mm 10 well comb (Wealtec).
- 12% SDS-PAGE resolving gel (0.75 mm); 2.31 ml ddH_2O, 2.8 ml 30 % Acrylamide/Bis (29:1) (Bio-Rad), 1.75 ml 1.5 M Tris-Cl, pH 8.8 (Sigma), 70 µl 10 % SDS (Bio-Rad), 70 µl 10 % APS (Bio-Rad), 2.8 µl TEMED (Bio-Rad)
- 5 % SDS-PAGE stacking gel (0.75 mm); 1.7 ml ddH_2O, 0.415 ml 30 % Acrylamide/Bis (29:1) (Bio-Rad), 0.315 ml 1 M Tris-Cl, pH 6.8 (Sigma), 25 µl 10 % SDS (Bio-Rad), 25 µl 10 % APS (Bio-Rad), 2.5 µl TEMED (Bio-Rad)
- 10X Tris-Glycine buffer (Sigma)
- V-GES Casting module (Wealtec)
- V-GES Electrode module and electrophoresis tank (Wealtec)
- Block Cooler (Wealtec)
- Block Heater (Wealtec)
- Glass plate separator (Wealtec)
- Coomassie Blue Staining Buffer (0.1 % Coomassie Blue R-250 (Bio-Rad) in Water/Methanol/Acetic acid (45:45:10))
- Destaining Buffer (Water/Methanol/Acetic acid (45:45:10))
- Dolphin-Doc image system with UV/White light convert plate (Wealtec)

PROCEDURE

Casting the gel and inserting into electrode module:

- Wipe off the glass plates and spacers with 75 % ethanol before casting. Open the casting module by pulling the wing releasers upwards. Set the module down in a

horizontal position. Arrange the glass plates and spacers sandwich into the casting module of V-GES in order (*fig. 2*). Remember to put the alignment card into the space between the thick glass plate and the U-shaped glass plate in order to make sure the spacers is inserted properly. Press the wing releasers downward in order to close the casting module. The alignment card should slide up and down without problems. (*fig. 5*)

- Prepare a 12% SDS-PAGE gel with 5 ml of the resolving gel solution (*fig. 6*) and then pipet 1 ml EtOH into the assembled casting module. Wait for the gel to polymerize for 60 minutes. While applying the solution into the space between the glass plate sandwiches, place the tip directly in between the glass plates. Slowly discharge the tip in order to minimize bubbles forming. After polymerization, remove the EtOH and pour in the prepared stacking gel solution on top of the polymerized resolving gel. Gently insert 0.75 mm 10 well comb into the space between the plates. Make sure there is no air bubbles trapped inside (*fig. 7*). Let the stacking gel polymerize for 60 minutes.
- Prepare the SDS-PAGE running buffer by diluting 100 ml 10 X buffer stock in 900 ml distilled water and add SDS for a final concentration of 3.5 mM.
- Release the gel sandwich from the casting module by applying equal force to both wing-releasers. Next, gently push the sandwich upwards through the two holes in the back of the casting module (*fig. 8*).
- Place the electrode module inside the vertical electrophoresis tank. Insert the gel sandwich into the electrode module by sliding it along the inner core of the module (*fig. 9*). After the gel sandwich is in place, close the door of the electrode module (*fig 10*). One-gel-gate may need to be inserted into the empty side of the module. Pour the SDS-PAGE running buffer into the space between the gel sandwich and the one-gel-gate up until app. 1 cm from the upper edge of the glass plates (*fig. 11*). Check to see if there is a leak. Then pour in 1 L of SDS-PAGE running buffer into the tank surrounding the gel assembly.

Sample preparation and loading:

- Thaw the frozen BSA (1 mg/ml) samples by inserting the tube into the Block Cooler (4 °C). Then add 25 μl of 4X protein loading dye into 75 μl thawed BSA solution.
- Boil the mixed BSA sample, Pre-stained marker, and ProMarker for 5 minutes in 95°C block heater prior to load the sample into the gel.

- Before loading samples onto the gel, thoroughly flush all the wells of the gel by gently pipetting running buffer up and down using a 100 µl pipette. Briefly spin down all samples before applying onto the gel. Apply 10 µl of protein samples into each well (*fig. 12*).
- Place the safety lid on top of the tank and connect the electrode wires to the power supply (*fig. 13*).
- Run the electrophoresis as follows; 90 V for 60 minutes and then 130V for another 60 minutes.
- After electrophoresis, remove the gel from the glass plates by inserting a glass plate separator between the glass plates and gently wiggle it until the plates separate (*fig. 14*). Remove the stacking gel and stain the resolving gel with Coomassie blue solution for 15 minutes.
- Destain the gel in destaining buffer with over night agitation.
- Thereafter, take a picture with Dolphin-Doc image system through the UV/White light convert plate.

RESULTS

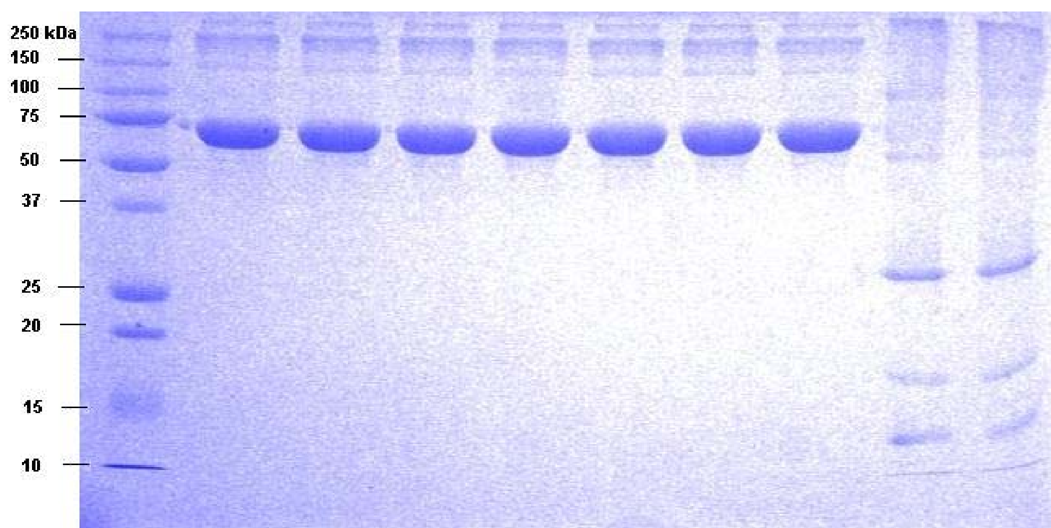

Figure 1. *The gel after electrophoresis. The outer left well contains a pre-stained marker and the two right outer lanes contain the pro-marker. The wells in between contain 1 mg/ml BSA. The stacking portion of the gel has been removed.*

REMARKS

- Separating with 12 % resolving gel and constant voltage-settings, BSA (66.2 kDa) was separated precisely at the right place compare to the Pre-stained Maker (Bio-Rad) migrations in the gel.
- While making the resolving gel, please level the gel with EtOH solution or distilled water after applying the solution into the glass plate. If not, the protein in the outer lanes will tend to lean toward one side.
- Different gel-percentage, separating times and voltage settings may be needed in order to distinguish different protein/peptides with small size or with extreme size. Besides adding additives, such as fluorescence protein stain, into protein sample, mixtures may also alter the migratory properties of proteins.
- Operators should be very careful during assembly of the glass plates and spacers. Wrong placement of spacers might cause leakage. Using the alignment card makes it easier to confirm the correct position of the spacers.
- After assembling the casting module, leakage can be tested prior to pouring the gel solution by pipetting water into the glass plate sandwich and waiting approximately 5 minutes.

APPENDIX

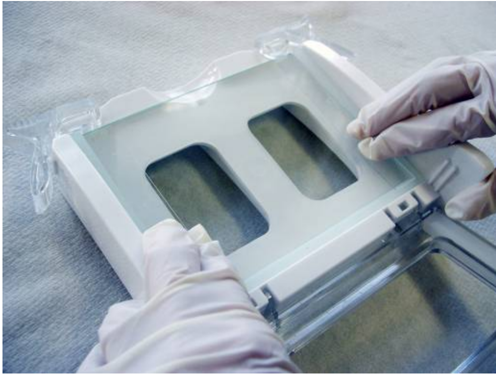

Figure 2. *While the casting module is in a horizontal position, place the spacers at both ends on top of the thick glass plate. Place an alignment card between the spacers and then put the u-shaped on top.*

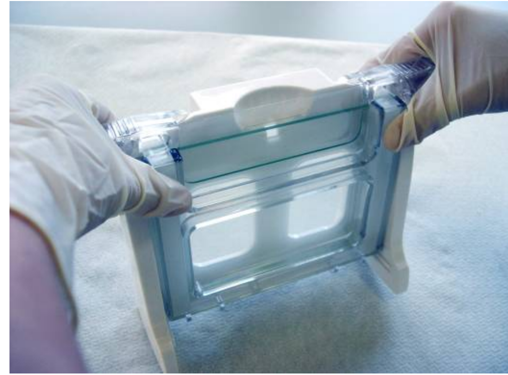

Figure 3. *Raise the module to a standing position and close the casting-door.*

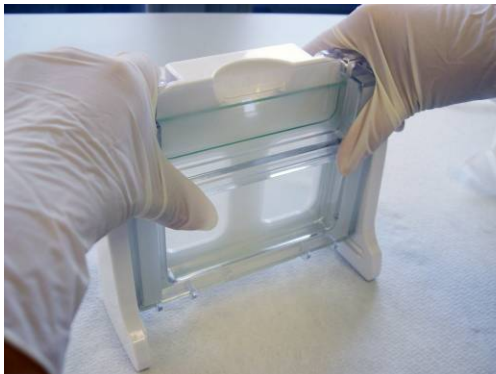

Figure 4. *Secure the door locking mechanism by applying equal force to the wing releasers and pressing down. To release the wind releasers, pull the wings upwards.*

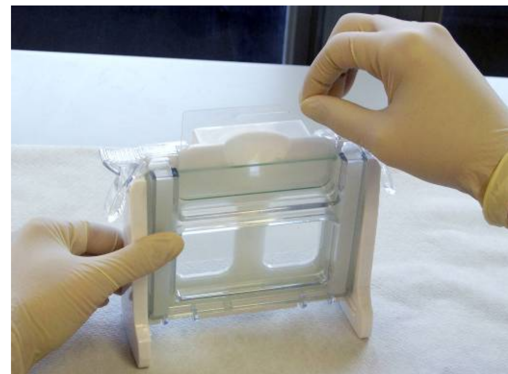

Figure 5. *Make sure the alignment card moves freely and that the spacers are in the correct position before you take it out.*

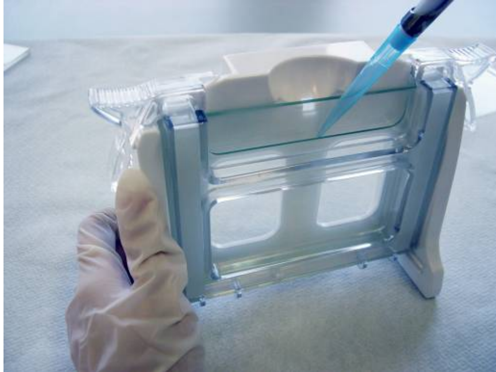

Figure 6. *Pour the resolving gel into the space between the glass plates. Place the pipette in the middle of the plates.*

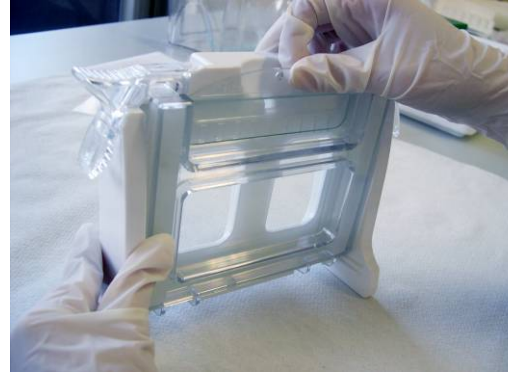

Figure 7. *After the stacking gel has been poured, gently insert the comb, making sure no air bubbles are trapped.*

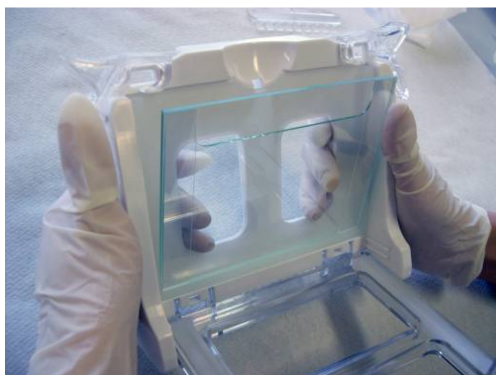

Figure 8. *Release the gel by grabbing your hands around the module. Press your fingers towards you through the holes in the back of the module while securing with your thumbs on the front side of the gel sandwich.*

Figure 9. *Insert the glass sandwich into the electrode module. Slide the sandwich closely along the pink silicone rubber on the middle part of the module making sure the inside of the gel sandwich risers.*

Figure 10. *Press together the doors of the electrode module. A clicking sound indicates that the doors are securely locked. When opening the lock, press down both sides of the white clamp-releasers (4 pcs) and slide the gel upwards.*

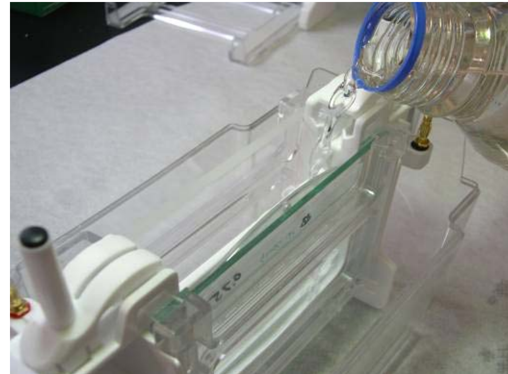

Figure 11. *Pour running buffer into the space between the glass plate sandwich and the one-gel gate up until 1 cm from the edge. Make sure the assembly does not leak before poring running buffer into the surrounding tank.*

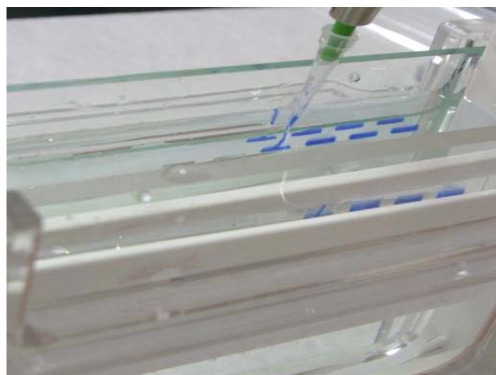

Figure 12. *Load your samples.*

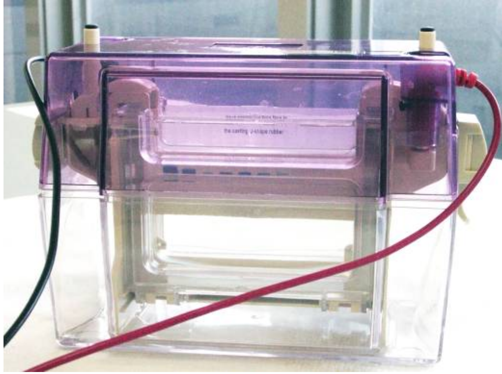

Figure 13. *Fasten the lid. The design of the assembly ensures the correct position of the lid.*

Figure 14. *Separate the glass plates using the glass plate separator. Wiggle carefully at one corner until the glass plates separate.*

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