Lab sheet #8

-SDS-Gel Polyacrylamide Electrophoresis-

-Objectives:

- To separate and calculate the molecular size of proteins by comparing the separated bands with known standard molecular weight marker.

-Method:

- Volumes of stacking gel and separation gel differ according to the thickness of gel casting:

Thickness of the gel	Vol. of stacking gel	Vol. of separation gel
0.75 mm	2 ml	4 ml
1.0 mm	3 ml	6 ml
1.5 mm	4 ml	8 ml

- **Volumes for a 10 ml separation gel:**

Acrylamide%	**6%**	**8%**	**10%**	**12%**	**15%**
H_2O	5.2 ml	4.6 ml	3.8 ml	3.2 ml	2.2 ml
Acrylamide/Bis-acrylamide (30% / 0.8% w/v)	2 ml	2.6 ml	3.4 ml	4 ml	5 ml
1.5 M Tris (pH=8.8)	2.6 ml	2.6 ml	2.6 ml	2.6 ml	2.6 ml
10% w/v SDS	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
10% w/v ammonium persulfate (APS)	100 µl	100 µl	100 µl	100 µl	100 µl
TEMED	10 µl	10 µl	10 µl	10 µl	10 µl

*Note: APS and TEMED must be added right before each use.

- **Volumes for a 5 ml stacking gel:**

H_2O	2975 µl
Acrylamide/Bis-acrylamide (30% / 0.8% w/v)	670 µl
0.5 M Tris-HCl (pH=6.8)	1250 µl
10% w/v SDS	50 µl
10% w/v ammonium persulfate (APS)	50 µl
TEMED	5 µl

- **5X Sample buffer (loading or disruption buffer):**

SDS	10% w/v
Tris-HCl (pH=6.8)	0.2 M
Dithiothreitol or betamercaptoethanol	10 mM
Glycerol	20% v/v
Bromophenol blue	0.05% w/v

*Make sure your target protein dissolved in the liquid phase, and no inappropriate ingredients are present (eg. Guanidine hydrochloride can interact with SDS and cause precipitate). Generally, to treat your unprepared sample, you can use a sonicator, lysis buffer, or both to sufficiently release your target protein released, and centrifuge to make supernatant and pellet separated.

- **1X Running buffer:**

SDS	0.1% w/v
Tris-HCl	25 mM
Glycine	200 mM

1. Make the separating gel:
    1. Set the casting frames (clamp two glass plates in the casting frames) on the casting stands.
    2. Prepare the gel solution (as described above) in a separate small beaker.
    3. Swirl the solution gently but thoroughly.
    4. Pipet appropriate amount of separating gel solution (listed above) into the gap between the glass plates.
    5. To make the top of the separating gel horizontal, fill in water (either isopropanol) into the gap until an overflow.
    6. Wait for 20-30 min to let it polymerize.

2. Make the stacking gel:
    1. Discard the water, and you can see the separating gel left.
    2. Pipette in the stacking gel until an overflow.
    3. Insert the well-forming comb without tapping air under the teeth. Wait for 20-30 min to let it polymerize.

3. Make sure a complete polymerization of the stacking gel and take out the comb. Take the glass plates out of the casting frame and set them in the cell buffer dam.
4. Pour the running buffer (electrophoresis buffer) into the inner chamber and keep pouring after overflow until the buffer surface reaches the required level in the outer chamber.

5. Preparing and loading the sample:
    1. Mix your samples with loading buffer (40 µl of protein sample + 10 µl of disruption buffer).
    2. Boil the mixture 3-5 min at 99 ℃.

3. Load prepared samples into wells (5-10 µl) and make sure not to overflow. Don't forget to load the protein marker in the first lane.

6. Running the gel:
    1. Cover the top and connect the anodes.
    2. Set an appropriate volt and run the electrophoresis when everything's done.
    3. Stop SDS-PAGE running when the downmost sign of the protein marker almost reaches the foot line of the glass plate. Generally, about 1 h for a 120 V and a 12% separating gel (for a separating gel possessing higher percentage of an acrylamide, the time will be longer).

7. Staining and de-staining the gel:
    1. Place the gel in the staining solution for 30-60 min, while gently shaking.
    2. Remove the gel and place it in the destaining solution for 1-2 h. Change the destaining solution 3-5 times until you can see clear bands with almost no blue background.

***General Notes:**

- Various factors affect the properties of the resulting gel.
- Higher concentration of APS and TEMED will lead to faster polymerization, on the other hand, lower stability and elasticity.
- The optical temperature for gel polymerization is 23-25 ℃. Low temperature will lead to turbid, porous, and inelastic gels.
- The pH is better to be neutral, and the gelation time should be limited to 20-30 min.

-Results:

- Picture of the gel.

Related questions:

1. To which electrode do the proteins migrate? Why?

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2. What is the relation between the protein size and migration?

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3. For what purpose was the SDS used?

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