2022 NEFU_China

Nucleic acid & protein electrophoresis

ABSTRACT

This protocol concludes two types of the electrophoresis used to detect target DNA or protein.

BEFORE STARTING

Prepare 50 x TAE, 1.5 mol/L Tris-Gly (pH 8.8), 1.0 mol/L Tris-Gly (pH 6.8), 5× Tris-Gly electrophoresis solution, coomassie blue staining solution, destaining solution before start.

1.Choose suitable electrophoresis method depends on the type of the sample.

1.2 Agarose gel electrophoresis

DNA was detected by agarose gel electrophoresis.

2.Weigh appropriate agarose depends on the concentration of the gel (1% agarose gel for detection and 1% or 2% for gel extraction).

3.Add 1X TAE to a conical flask. Need to prepare 1X TAE with 50X TAE.

Extra 2~3ml of 1X TAE was strongly recommended to be added to avoid the reduction of solution during heat.

4.Heat up by microwave until the solution is homogeneous.

5.Cool at room temperature for 3~5 min.

6.Add 1 μl Gold view into solution and mix well when the solution is about 60℃.

7.Put the solution into bed for polymerize, make sure "comb" is well placed and the solution is balanced.

8.Wait about 20 min to let the gel completely concretes.

9.Mix the sample with 6 X orange loading buffer and load the sample into the sample holes.

Remember to load the DNA marker to the sample hole.

10.Put the bed with gel into the electrophoresis chamber.

11.Set the voltage of electrophoresis (80V~150V) and begin to run.

12.Stop running when the front indicator reach about 3/4 length of the gel.

13.Use https://www.bio-rad.com/en-us/product/gel-doc-xr-gel-documentation-system?ID=O494WJE8Z
to observe the gel.

1.2 SDS-PAGE

Protein was detected by SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis)

Sample preparation

2.Centrifuge 6OD bacteria in 1.5 ml eppendorf (EP) tube at 4,200 x rpm for 5 minutes at room temperature.

3.Decant or aspirate and discard the culture media.
4.Use 600µl ddH2O spirate and dis completely resuspend cell pellet.
5.Put the EP tube on the ice and use ultrasonic cell disruptor to disrupt cells until the system become clear.
6.Centrifuge at 10,000 x rpm for 10 minute at room temperature.
7.Take 50 µl of supernatant into another EP tube and abandon other supernatant.
8.Completely aspirate residual supernatant.
9.Use 600µl ddH2O spirate and dis completely resuspend sediment.
10.Take 50 µl of resuspending into another EP tube.
11.Add 10µl 6×Protein loading into each EP tube and mix thoroughly.
12.Store sample at -20℃.

Gel preparation

13.It is necessary to were latex gloves and mask when prepare the gel because most of the reagent are toxic.
All the things used in the next steps should be washed after use and can not be touched without wearing gloves.
Disposable plastic gloves are not allowed to use.
14.Wash glass plates and install the casting stand with the glass plates.
15.Inject the water into the pouring trough to check leakage. The liquid level will drawdown in few minutes if the pouring trough has leakage in it.
16.Pour out the water completely and make sure the pouring trough is dry.
17.Prepare the separation gel (12%) by mix ingredients below:

	A	B
1	ddH2O	3.3ml
2	30% Acr- Bis(29:1)	4ml
3	1.5 mol/L Tris-Gly (pH 8.8)	2.5ml
4	10% SDS	100µl
5	10% Ammonium persulfate	100µl
6	TEMED	4µl

Make sure that add TEMED at last and stir the gel fast to avoid too early concretion of the gel.

18.Inject the separation gel into the pouring trough quickly and inject water to full the trough.
There is no need to worry about the bubbles during injection because the water can eliminate most of them.
19.Wait about 30 min to let the gel concrete.

20.Pour out the water completely.
21.Prepare the spacer gel (5%) by mix ingredients below:
Prepare the spacer gel (5%) by mix ingredients below:

	A	B
1	ddH2O	3.4ml
2	30% Acr- Bis(29:1)	830μl
3	1.0 mol/L Tris-Gly (pH 6.8)	630μl
4	10% SDS	50μl
5	10% Ammonium persulfate	50μl
6	TEMED	5μl

Make sure that add TEMED at last and stir the gel fast to avoid too early concretion of the gel.
22.Inject the separation gel into the pouring trough quickly to full the trough.
23.Insert the comb into pouring trough.
24.Wait about 30 min to let the gel concrete.
25.Pull the comb out carefully.

Electrophoresis

26.Install the electrophoresis pool and pour the diluted 5× Tris-Gly electrophoresis solution (Tris-Base 15.1g, Glycine 94g, SDS 5g, pH=8.3) into the pool to the appropriate position.
27.Load the sample (20μl）and protein marker (5μl) into the sample holes.
28.Install the gel with glass plates into the electrophoresis pool.
29.Run the gel at the voltage of 80V for about 30min and change the voltage to 120V and run the gel until the front indicator run out of the gel.
30.Stop running and Peel the gel into the plastic box and clean it with distilled water (ddH2O).

Staining

31.Add proper amount of coomassie blue staining solution (400ml, coomassie bright blue r-250 0.4g, isopropyl alcohol 100ml, glacial acetic acid 40ml, ddW260ml, filtered) .
32.Heat by microwave until almost boiling (moderate for about 1min30s).
33.Use the flat table to shake the plastic box **30 rpm, Room temperature , 00:30:00**

34.Recycle the staining solution to another bottle and wash the gel using ddH2O.

DeStaining

35.Add destaining solution (500ml, glacial acetic acid 50ml, anhydrous ethanol 75ml, distilled water 375ml).
36.Heat by microwave until almost boiling (moderate for about 1min30s).
37.Use the flat table to shake the plastic box **30 rpm, Room temperature , 00:20:00**
38.Change the destaining solution and continue to shake until decolor completely.

Observation

39.Use https://www.bio-rad.com/en-us/product/gel-doc-xr-gel-documentation-system?ID=O494WJE8Z
to observe the gel.