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Nucleic acid & protein electrophoresis V.2

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ABSTRACT

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

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MATERIALS TEXT

Agarose electrophoresis:
Microwave
Electrophoresis apparatus
Electrophoresis pool
Gel bed
DdH2O
Agarose
50X TAE
Gold view
6 X orange loading buffer

SDS-PAGE:
Microwave
Centrifuge
Electrophoresis apparatus
Electrophoresis pool
Glass plate
casting stand
ddH2O
30% Acr- Bis(29:1)
1.5 mol/L Tris-Gly (pH 8.8)
10% SDS
10% Ammonium persulfate
TEMED
1.0 mol/L Tris-Gly (pH 6.8)
5× Tris-Gly electrophoresis solution (Tris-Base 15.1g, Glycine 94g, SDS 5g, pH=8.3)
coomassie blue staining solution (400ml, coomassie bright blue r-250 0.4g, isopropyl alcohol 100ml, glacial acetic acid 40ml, ddW260ml, filtered)
Destaining solution (500ml, glacial acetic acid 50ml, anhydrous ethanol 75ml, distilled water 375ml).

SAFETY WARNINGS

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.

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.
Step 1 includes a Step case.
Agarose gel electrophoresis
SDS-PAGE

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°C.

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

Gel Doc XR+ Gel Documentation System
Gel Documentation System
Bio-rad Laboratories **1708195**

to observe the gel.