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SOP-100

SDS – PAGE Analysis

Procedure for resolving protein molecules in polyacrylamide gels.

Section 1

Materials and Reagents

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1.5 M Tris-HCl (pH 8.8)

Tris base 181.7 g in 1 L H_2O, adjust pH to 8.8 using HCl.

0.5 M Tris-HCl (pH 6.8)

Tris base 60.6 g in 1L H_2O, adjust pH to 6.8 using HCl.

10 % (w/v) SDS

SDS (sodium dodecal sulfate) 100g in 1L H_2O.

2X SDS Sample Buffer

0.25 M Tris Hcl (pH 6.8)

4 % (w/v) SDS

20 % (v/v) glycerol

trace bromphenol blue

Note: For reducing conditions use 950 ul 2x SDS sample buffer plus 50 2-mercaptoethanol.

10 % (w/v) Ammonium Persulfate (APS)

10 g ammonium sulfate in H_2O, aliquot into 1 ml vials and store at –20 C until needed.

TEMED

Acrylamide/bisacrylamide stock solution

For 1 L

30 % (w/v) acrylamide 300 g

0.8 % (w/v) bisacrylamide 8 g

Add H_2O to final volume and filter through 0.22 um membrane. Store at 4 C in the dark.

Section 2

Protocol

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1. Preparing the sample

Add equal volume of 2 x SDS sample buffer to sample. Heat in boiling water bath (100 C) for 5 min. Cool samples to room temperature and give brief spin to pellet insoluble material.

2. Casting the gels.

Wash all glassware. Assemble gel apparatus. Mix the lower resolving gel:

Lowere Resolving gel	> 50 kD	30-40 kD	20 –30 kD	<20 kD
% Acrylamide	8%	10 %	12 %	15 %
Acrylamide / bisacrylamide solution	2.13 ml	2.67 ml	3.20 ml	4.00 ml
H_2O	3.87 ml	3.33 ml	2.80 ml	2.00 ml
1.5 M Tris-HCl (pH 8.8)	2.00 ml	2.00 ml	2.00 ml	2.00 ml
10 % SDS	80 ul	80 ul	80 ul	80 ul
10 % APS	45 ul	45 ul	45 ul	45 ul
TEMED	12 ul	12 ul	12 ul	12 ul
Final Volume	8 ml	8 ml	8 ml	8 ml

Add the APS and TEMED last, mix gently but quickly. Pour solution into casting apparatus until height of liquid is about 1 cm from the top of the gel plates. Overlay with H_2O and allow to polymerize for about 1 hr.

Prepare stacking gel:

Stacking Gel	3 %
Acrylamide / bisacylamide solution	0.75 ml
H_2O	3.00 ml
0.5 M Tris-HCl (pH 6.8)	1.25 ml
10 % SDS	50 ul
10 % APS	30 ul
TEMED	8 ul
Final Volume	5.00 ml

After the resolving gel has polymerized pour off the upper liquid. Rinse the upper portion of the gel with water. Rinse quickly with stacking gel solution and then fill uper chamber with stacking gel solution. Insert comb between plates. The upper gel should polymerize in about ten minutes.

3. Load the protein samples

Mount plates into electrophoresis chamber, add running buffer and then remove comb from gels.

Load the protein samples into the wells through the running buffer. Take care that the samples do not cross contaminate wells.

4. Electrophoresis the gel.

Attach the electric leads to the power supply, black to negative and red to the positive pole. Proteins bound with SDS are negatively charged at pH 8.3 and will migrate toward the positive electrode.

Run the stacking gel at 8 mA (constant current) per gel. If two gels are running simultaneously in one apparatus, run the gels at 16 mA. The proteins will form a narrow band at the stacking / resolving gel interface.

When the bromphenol blue enters the resolving gel increase the current to 18 mA per gel. Stop the power when the bromphenol blue reaches the botton of the resolving gel. Do not let the dye run out of the bottom.

Section 3

References

References

Short Protocols in Molecular Biology. 4th Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999