Polyacrylamide Gel System For Electrophoresis Of Proteins

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Abstract

Citation: David Dunigan and Irina Agarkova Polyacrylamide Gel System For Electrophoresis Of Proteins. **protocols.io** dx.doi.org/10.17504/protocols.io.ep7bdrn
Published: 13 Jun 2016

Guidelines

GEL PREPARATIONS

Sample Recipe for Resolving Gel:

12.5% acrylamide (40.0 mL volume)
16.7 mL 30% acrylamide solution
10.0 mL 4X resolving gel buffer
12.4 mL d-H_2O
0.4 mL 10% SDS
20.0 µL TEMED
0.5 mL 10% Ammonium persulfate (APS) - make fresh

For gradient gels, a 4 M to 8 M urea gradient is included in the gel to stabilize the acrylamide gradient and to aid in maximal denaturation of the polypeptides.

Remember that when making volume calculations that 1.0 gm of urea is equivalent to 0.76 mL of d-H_2O. To achieve an acrylamide concentration greater than about 12% in the 8 M urea, the concentration of the resolving gel buffer or the acrylamide stock solution needs to be increased. If heat is used to get the 8 M urea into solution remember to let the solution cool to room temperature before pouring the gradient.

The amount of TEMED and ammonium persulfate are reduced when pouring a gradient gel compared to a straight percentage gel to allow ample time for pouring the gradient before polymerization of the gel.

Sample Recipe for Gradient Gel:

(for 250 mm gel, 1.5 mm spacers)

7.5% Acrylamide 4 M Urea	**15.0% Acrylamide 8 M Urea**

30.0% acrylamide	6.3 mL	12.5 mL
4X resolving buffer	6.25 mL	---
10X resolving buffer	---	2.5 mL
Urea	6.0 gm	12.0 gm
10% SDS	0.25 mL	0.25 mL
d-H_2O	7.5 mL	0.55 mL
TEMED	7.5 µL	7.5 µL
10% APS	87.0 µL	87.0 µL

Mix all of the ingredients together except the ammonium persulfate. Don't add the ammonium persulfate until just before pouring the gel.

Gently overlay the gel with d-H_2O (or 1X resolving gel buffer) and allow the gel to polymerize. The line between the gel and the water will first fade away and then become very sharp when the gel has polymerized.

Sample Recipe for Stacking Gel

(for 250 mm gel, 1.5 mm spacers)

	4.5% Acrylamide No Urea	**4.5 % Acrylamide 4 M Urea**
Urea	---	2.4 gm
30.0% acrylamide	1.5 mL	1.5 mL
4X stacking gel buffer	2.5 mL	2.5 mL
10% SDS	0.1 mL	0.1 mL
d-H_2O	5.8 mL	3.9 mL
TEMED	5.0 µL	5.0 µL
10% APS	130.0 µL	130.0 µL

Pour the stacking gel immediately after adding the ammonium persulfate. Insert the gel comb to form the wells. Allow the stacking gel to polymerize for 60 min before use.

Boil the samples in the cracking buffer 5-10 min before loading onto the gel.

Electrophorese the gel at 100 volts (constant) or 10 milliamps (constant) until the dye front just runs off the gel.

MINIGELS

Resolving Gel Recipes:

5.0 mL gel

	9.0%	**10.0%**	**12.5%**	**15.0%**
30.0% acrylamide	1.50 mL	1.67 mL	2.10 mL	2.50 mL
4X resolving gel buffer	1.25 mL	1.25 mL	1.25 mL	1.25 mL
20% SDS	25.0 µL	25.0 µL	25.0 µL	25.0 µL
d-H2O	2.25 mL	2.08 mL	1.65 mL	1.25 mL
10% APS	62.5 µL	62.5 µL	62.5 µL	62.5 µL
TEMED	2.5 µL	2.5 µL	2.5 µL	2.5 µL

Stacking Gel Recipes:

2.5 mL gel

	3.0%	**3.5%**	**4.5%**	**5.0%**
30.0% acrylamide	0.25 mL	0.292 mL	0.375 mL	0.417 mL
4X stacking gel buffer	0.625 mL	0.625 mL	0.625 mL	0.625 mL
20% SDS	12.5 µL	12.5 µL	12.5 µL	12.5 µL
d-H_2O	1.625 mL	1.583 mL	1.45 mL	1.458 mL
10% APS	32.5 µL	32.5 µL	32.5 µL	32.5 µL
TEMED	1.25 µL	1.25 µL	1.25 µL	1.25 µL

COOMASSIE STAINING OF THE GELS

Stain the gels with coomassie blue stain according to the following recipe:

1.0 gm Coomassie Brilliant Blue R

455 mL methanol

455 mL d-H_2O

90 mL glacial acetic acid

Gels are destained with the following:

700 mL methanol

200 mL glacial acetic acid

2600 mL d-H_2O

Stain and destain the gels at 65^{0}C. Small foam rubber pieces added during the destaining process absorb some of the coomassie with the result that less destain is needed and the destain need not be changed as often.

Protocol

Stock Solutions

Step 1.
Prepare 30.0% Acrylamide stock solution (30.0:0.8).

PROTOCOL

. 30.0% Acrylamide stock solution (30.0:0.8)

CONTACT: Irina Agarkova

Step 1.1.
Add 60.0 gm of acrylamide and 1.6 gm of N, N'-methylenebisacrylamide (bisacrylamide) to 140 mL of d-H_2O.

Step 1.2.
Stir until dissolved.

Step 1.3.
Adjust the volume to 200 mL.

Step 1.4.
Store protected from the light.

Stock Solutions

Step 2.
Prepare Resolving gel buffer (4X).

PROTOCOL

. Resolving gel buffer (4X)

CONTACT: Irina Agarkova

Step 2.1.
Dissolve 45.4 gm of Tris base in 200 mL of d-H_2O.

Step 2.2.
Adjust the pH to 8.8 with concentrated HCl.

Step 2.3.
Adjust to a final volume of 250 mL.

Stock Solutions

Step 3.
Prepare Stacking gel buffer (4X).

PROTOCOL

. Stacking gel buffer (4X)

CONTACT: Irina Agarkova

Step 3.1.
Dissolve 6.1 gm of Tris base in 90 mL of d-H_2O.

Step 3.2.
Adjust the pH to 6.8 with concentrated HCl.

Step 3.3.
Adjust to a final volume of 100 mL.

Stock Solutions

Step 4.
Prepare Running gel buffer (10X).

PROTOCOL

. Running gel buffer (10X)

CONTACT: Irina Agarkova

Step 4.1.
Combine 45.5 gm of Tris base and 216.0 gm of Glycine.

Step 4.2.
Adjust the final volume to 1500 mL.

NOTES

Irina Agarkova 22 Mar 2016
The pH should be approximately 8.3 without adjustment.

Step 4.3.
SDS and 2-mercaptopropionic acid are added to the buffer prior to electrophoresis: the SDS to

0.1% and 1 mL of mercaptopropionic acid to 1 liter of 1X running buffer.

Stock Solutions

Step 5.
Prepare Cracking buffer, 4X (10 mL).

PROTOCOL

. Cracking buffer, 4X (10 mL)

CONTACT: Irina Agarkova
Step 5.1.

Combine:

5.0 mL of 20% SDS
2.0 mL of 100% Glycerol
1.0 mL of 1% Bromophenolblue
2.0 ml of 4X stacking gel buffer
0.5 mL of 500 mM DTT

Step 5.2.

For solid samples, dilute the cracking buffer to 1X concentration and use to suspend samples. Boil samples for 10 minutes before loading onto gel.

Step 5.3.

For aqueous samples, add 1.0 µL of cracking buffer per 3.0 µL of sample and boil samples for 10 minutes before loading onto gel.

Gel Preparations: Sample Recipe for Resolving Gel

Step 6.
See guidelines for Sample Recipe for Resolving Gel.

Gel Preparations: Sample Recipe for Gradient Gel

Step 7.
See guidelines for ingredients.

Gel Preparations: Sample Recipe for Gradient Gel

Step 8.
Mix all of the ingredients together except the ammonium persulfate.

NOTES

Irina Agarkova 24 Mar 2016
Don’t add the ammonium persulfate until just before pouring the gel.

Gel Preparations: Sample Recipe for Gradient Gel

Step 9.

Gently overlay the gel with d-H_2O (or 1X resolving gel buffer) and allow the gel to polymerize.

NOTES

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The line between the gel and the water will first fade away and then become very sharp when the gel has polymerized.

Gel Preparations: Stacking Gel Recipe

Step 10.

See guidelines for ingredients.

Gel Preparations: Stacking Gel Recipe

Step 11.

Pour the stacking gel immediately after adding the ammonium persulfate.

Gel Preparations: Stacking Gel Recipe

Step 12.

Insert the gel comb to form the wells.

Gel Preparations: Stacking Gel Recipe

Step 13.

Allow the stacking gel to polymerize for 60 min before use.

DURATION

01:00:00

Gel Preparations: Stacking Gel Recipe

Step 14.

Boil the samples in the cracking buffer 5-10 min before loading onto the gel.

DURATION

00:10:00

Gel Preparations: Stacking Gel Recipe

Step 15.

Electrophorese the gel at 100 volts (constant) or 10 milliamps (constant) until the dye front just runs off the gel.

Minigels

Step 16.

See guidelines for Resolving Gel Recipes and Stacking Gel Recipes.

Coomassie Staining of The Gels

Step 17.

Stain the gels with coomassie blue stain according to the following recipe:

1.0 gm Coomassie Brilliant Blue R
455 mL methanol
455 mL d-H_2O
90 mL glacial acetic acid

Coomassie Staining of The Gels

Step 18.

Gels are destained with the following:

700 mL methanol
200 mL glacial acetic acid
2600 mL d-H_2O

Coomassie Staining of The Gels

Step 19.

Stain and destain the gels at 65°C.

Coomassie Staining of The Gels

Step 20.

Small foam rubber pieces added during the destaining process absorb some of the coomassie with the result that less destain is needed and the destain need not be changed as often.