Casting of a polyacrylamide gel

Brian Kenn Baltzar.

Abstract

A protocol to cast a polyacrylamide gel.

Used in for iGEM SDU 2016

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Guidelines

20% Separation gel-mix 15 mL

10 mL	30% Acrylamide/bis	
3.75 mL	1.5M Tris-HCl, pH 8.8	
150 µL	10% SDS	
1.03 mL	diH2O	
7.5 µL	TEMED	ADD JUST BEFORE CASTING!
75 µL	10% APS	ADD JUST BEFORE CASTING

4% Stacking gel-mix 15 mL

1.98 mL	30% Acrylamide/bis	
3.78 mL	0.5M Tris-HCl, pH6.8	
150 µL	10% SDS	
9 mL	diH2O	
15 µL	TEMED	ADD JUST BEFORE CASTING!
75 µL	10% APS	ADD JUST BEFORE CASTING

X% Separation gel-mix 15 mL

0.5 * X mL	30% Acrylamide/bis	
3.75 mL	1.5M Tris-HCl, pH 8.8	
150 µL	10% SDS	
11.03-(0.5*X) mL	diH2O	
7.5 µL	TEMED	ADD JUST BEFORE CASTING!
75 µL	10% APS	ADD JUST BEFORE CASTING

2x SDS-PAGE Buffer

3.75 mL	Tris-HCl pH 6.8, 0.5M
24.0 mL	Glycerol
300 µL	1.0% Bromophenol blue eller xylene cyanol (MEGET Lidt)
6.0 mL	10% SDS
-> 30 mL	diH_2O

Acrylamide/Bis 30%

87.60 g	Acrylamide (29.2g / 100mL)
2.40 g	N'N'-bis-methylene-acrylamide
-> 300 mL	diH_2O
Filter and store at 4ºC	

1.5 M Tris-HCl, pH 8.8 (150 ml)

27.23 g	Tris-base (18.15 g/100 ml)

80 mL	diH_2O
Adjust to pH 8.8 with 6N HCl	
-> 150 mL	diH_2O
store at 4ºC	

0.5 M Tris-HCl, pH 6.8

6 g	Tris-base (18.15 g/100 ml)
60 mL	diH_2O
Adjust to pH 6.87 with 6N HCl	
-> 100 mL	diH_2O
store at 4ºC	

10% APS

0.10 g	Ammonium persulfate
1 mL	diH_2O

10% SDS

10.00 g	SDS
60 mL	diH_2O
Dissolve with gentle stirring	
-> 100 mL	diH_2O

Protocol

Step 1.

Wash all of the hardware - make sure they are clean otherwise it may leak

Step 2.

Assemble the glass plates and spacers and fix them in the plastic mounting devices.

Step 3.

Mount the assembled glassplates on the casting stand and make sure the stand is level

Step 4.

Separation gel.

Step 5.

Add the APS to the Separation gel-mix

Step 6.

Step 7.

Step 8.

Step 9.

When the remaining gel-mix in the falcon tube has polymerized, remove the H_2O from the top of the gel by tilting the whole assembly and using a napkin to remove the excess H_2O.

Step 10.

Step 11.

Mix by gently inverting a few times

Step 12.

Add the TEMED - Cast the gel immediately after addition of TEMED up to the top.

Step 13.

Insert the comb onto the top of the gel.

Step 14.

Warnings

Remember to wear gloves as Acrylamide/Bis is very toxic.