LowC SDS-PAGE

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Abstract

SDS-PAGE using gels with low crosslinking of acrylamide and bisacrylamide. This protocol can be used to separate phosphorylation forms of KaiC proteins.

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Guidelines

references:

1. SDS-PAGE modified from

Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage 780 t4. *Nature 1970*, 227(5259):680–5,

and

Green MR and Sambrook J: Molecular cloning: a laboratory manual - 4th ed. *Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2012*

2. low C content according to

Nishiwaki T, Satomi Y, Kitayama Y, Terauchi K, Kiyohara R, Takao T, Kondo T: A sequential program of dual phosphorylation of KaiC as a basis for circadian rhythm in cyanobacteria. *Embo J* 2007, 26:4029-4037.

Possible modifícations:

The total acrylamide concentration (%T) and crosslinking of acrylamide and bis-acrylamide (%C) can be adjusted using the following formula (provided by Carl Roth

https://www.carlroth.com/downloads/ba/en/3/BA_3037_EN.pdf)

Vt = total gel volume (ml)

T = total gel concentration in % = % Acrylamide + % Bisacrylamide

C = % Crosslinking = (% Bisacrylamide x 100) / T

Va = Volume Gel A in ml

Vb = Volume Gel B in ml

Va = (T x (100 - C) x Vt) / 3000

Vb = (T x C x Vt) / 200

required chemicals/buffers

chemical/buffer	comments
Rotiphorese® Gel A	see materials tab
Rotiphorese® Gel B	see materials tab
1.5 M Tris/HCl pH8.8	
1 M Tris/HCl pH 6.8	
TEMED	
10 % (w/v) ammonium persulfate (APS)	
20 % SDS (w/v)	
2-propanol	
1 x Tris-Glycine elctrophoresis buffer	see buffer table
5 x SDS Gel loading buffer	see buffer table
Rotiphorese® Blau R	see materials tab
7 % acetic acid	
Destaining solution	see buffer table

buffer compositions:

name	ingredients	reference

1 x Tris-Glycine elctrophoresis buffer	250 mM Tris 0.1 % (w/v) SDS 0.192 M Glycin	Green and Sambrook (2012)
5 x SDS Gel loading buffer	250 mM Tris-HCl, pH 6,8 40 % (v/v) glycerol 8 % (w/v) SDS 100 mM DTT 0.1 % (w/v) bromophenol blue	Green and Sambrook (2012)
destaining solution	acetic acid: ethanol: H_2O 10:50:40 (v/v/v)	

required equipment:

heating block
glass plates, spacers, combs, gel casting unit
gel chamber
syringe
power supply
benchtop centrifuge
horizontal shaker

Materials

Rotiphorese® Gel A 3037.2 by Carl Roth
Rotiphorese® Gel B 3039.2 by Carl Roth
Rotiphorese® Blau R 3074.1 by Carl Roth

Protocol

gel preparation
Step 1.

Clean glass plates, spacers, combs and all components with 70 % ethanol and assemble gel casting unit.

gel preparation
Step 2.

Place the comb between the glass plates and mark a line ca. 0.5 cm below the comb. Take out the comb again.

gel preparation

Step 3.

To prepare a 0.67 % C resolving gel mix the following ingredients:

Depending on the gel chamber, the volume has to be adjusted. You can adjust % T using the formula given in additional information.

For 10 ml	**10 % T, 0.67 %C**	**11 % T, 0.67 %C**	**13 % T 0.67 %C**
H2O	3.8 ml	3.44 ml	2.71
1.5 M Tris/HCl pH 8.8	2.5 ml	2.5 ml	2.5 ml
Rotiphorese® Gel A	3.31	3.64 ml	4.3 ml
Rotiphorese® Gel B	335 µl	370 µl	440 µl
20 % SDS	50 µl	50 µl	50 µl
TEMED	5 µl	5 µl	5 µl

For 20 ml	10 % T, 0.67 %C	11 % T, 0.67 %C	13 % T 0.67 %C
H2O	7.6 ml	6.88 ml	5,3
1.5 M Tris/HCl pH 8.8	5 ml	5 ml	5 ml
Rotiphorese® Gel A	6.62 ml	7.28 ml	8,6 ml
Rotiphorese® Gel B	670 µl	740 µl	880 µl
20 % SDS	100 µl	100 µl	100 µl
TEMED	10 µl	10 µl	10 µl

For 30 ml	**10 % T, 0.67 %C**	**11 % T, 0.67 %C**	**13 % T 0.67 %C**
H2O	11.4 ml	10.32 ml	7.95
1.5 M Tris/HCl pH 8.8	7.5 ml	7.5 ml	7.5 ml
Rotiphorese® Gel A	9.93	10.92 ml	12.9 ml
Rotiphorese® Gel B	1 ml	1.11 ml	1.32 ml
20 % SDS	150 µl	150 µl	150 µl
TEMED	15 µl	15 µl	15 µl

gel preparation

Step 4.

Pour a stop gel for each gel: take a 1 ml aliquot, add 40 µl APS, and immediately pour between the glass plates. Wait until the stop gel is polymerized.

gel preparation

Step 5.

Add 50 µl APS to the residual gel mixture (100 µl for 20 ml total gel volume, 150 µl for 30 ml total volume), mix gently, immediately pour resolving gel between the glass plates and overlay with 0.5 ml 2-propanol. Wait until separating gel is polymerized.

gel preparation

Step 6.

Pour of 2-propanol and dry carefully using a sheet of thin filter paper.

gel preparation

Step 7.

Mix the following ingredients (5 % T, 2.7 % C stacking gel):

Depending on the gel chamber, the volume has to be adjusted.

For 3 ml	**5 % T, 2.7 %C**
H2O	1.89 ml
1 M Tris/HCl pH 6.8	0,38 ml
Rotiphorese® Gel A	0.49 ml
Rotiphorese® Gel B	0.2 ml
20 % SDS	15 µl
TEMED	3 µl

For 6 ml	**5 % T, 2.7 %C**
H2O	3.77
1 M Tris/HCl pH 6.8	0.75 ml
Rotiphorese® Gel A	1 ml
Rotiphorese® Gel B	0.4 ml
20 % SDS	30 µl
TEMED	6 µl

For 12 ml	**5 % T, 2.7 %C**
H2O	7.56 ml
1 M Tris pH 6.8	1.5 ml
Acrylamid A	1.96 ml
Acrylamid B	0.8 ml
20 % SDS	60 µl
TEMED	12 µl

gel preparation

Step 8.

Add 30 µl APS to the gel mixture (60 µl for 6 ml total gel volume, 120 µl for 12 ml total volume), mix gently, immediately pour between the glass plates on top of the resolving gel and place the comb between glass plates into the stacking gel. Wait until the stacking gel is polymerized.

*If you use peqLab Twin ExW System, it will be better to place the combs **before** pouring the gel*

sample preparation

Step 9.

Mix your protein sample with 5 x SDS Gel loading buffer (1x final concentration). For separation in mini gels use 0.75–3 µg of recombinant KaiC protein (55 kDa).

sample preparation

Step 10.

Denature for 3 min at 95 °C.

sample preparation

Step 11.

Spin down for 1 min at max. speed using a benchtop centrifuge.

electrophoresis

Step 12.

Remove comb and wash wells thoroughly with MilliQ using a syringe. Place gels in a gel chamber and fill with running buffer.

electrophoresis

Step 13.

Load samples into the bottom of the wells.

electrophoresis

Step 14.

Connect gel chamber with power supply and separate proteins at 120 V. When samples enter the separating gel, set voltage to 150-180 V.

staining

Step 15.

Disassemble gel chamber. Carefully separate glass plates and place gels in a plastic box with MiliQ.

staining

Step 16.

Incubate gel in destaining solution for 10 min to fixate proteins and remove SDS (*SDS interferes with Coomassie Blue staining*) under gentle agitation on a horizontal shaker.

staining

Step 17.

Pour off the water and incubate in Rotiphorese® Blau R for 10-30 min under gentle agitation. Use a lid to avoid evaporation of methanol.

staining

Step 18.

Remove Rotiphorese® Blau R (*can be reused!!!*) and rinse with MiliQ.

staining

Step 19.

Discard MiliQ and alternate incubation in destaining solution and 7 % acetic acid until the background of the gel is clear. You can add a tissue to soak up Coomassie.

Warnings

Always wear vinyl gloves, when handling acrylamide!

Discard staining solution (contains methanol and Coomassie blue) and destaining solutions (contains ethanol and acetic acid) in the appropriate liquid waste!