Oct 18, 2019

SDS-PAGE

DOI
dx.doi.org/10.17504/protocols.io.8evhte6

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DOI: https://dx.doi.org/10.17504/protocols.io.8evhte6

Protocol Citation: Anna Behle, Anika Wiegard 2019. SDS-PAGE. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.8evhte6

Protocol status: Working
We use this protocol and it's working

Created: October 18, 2019

Last Modified: October 18, 2019

Protocol Integer ID: 28853

Keywords: page protocol for sds page gel, sds page gel, page protocol, sd, blue dye, coomassie blue dye, gel, buffer, protocol

Abstract

Protocol for SDS PAGE gel, buffers and Coomassie blue dye.

Materials

- Acrylamid
- 48 g Acrylamid, 1,5 g Bisacrylamid ad 100 ml H2O

- Gel buffer
- 3 M Tris/HCl pH 8,45, 0,3% SDS

- Cathode buffer
- 100 mM Tris, 100 mM Tricin, 0,1% SDS, pH8,25

- Anode buffer
- 100 mM Tris/HCl, pH 8,9

- Fixation solution
- 50% Ethanol, 10% Acetate, 40% H2O

- Dyeing solution
- Coomassie Blue

- Gel chambers (0,8mm, PerfectBlue Double gel system Twin ExW, gel size 20 × 10 cm)

Troubleshooting

Safety warnings

Acrylamid is a neurotoxin, always wear gloves while working with it before polymerization.

Preparing and pouring the SDS gel

1 ,Mix together all components except for APS and TEMED first. Make sure the Urea is completely dissolved by moving the solution at 150 rpm for about 15 minutes. To make the pockets in the collection gel more visible, use gel buffer with added bromphenole blue dye.

	Component	Running gel	Collection gel
	Urea	4,8 g	-
	Acrylamid	2,9 ml	0,7 ml
	Gel buffer	4,7 ml	1,53 ml
	H2O	3,8 ml	4 ml
	APS	88 µl	100 µl
	TEMED	10 µl	10 µl

Components of an SDS gel, add APS and TEMED only after all other components are mixed and urea has completely dissolved.

2 Mix 1 ml of running gel (without APS and TEMED) with 2 µl TEMED and 20 µl APS to create a quickly-polymerising stop gel. Quickly pipette into gel chamber until the bottom of the chamber is filled evenly. This will prevent the slower hardening running gel form leaking out of the chamber.

3 Add APS and TEMED to the running gel and mix by inverting the tube. Pipette ca. 5 ml in to the gel chamber and pipette ca. 500 µl of Isopropanol on top of the running gel to exclude air.

4 When the running gel is fully polymerised, remove all isopropanol by pouring it out and then drying the corners of the gel with some absorbant paper.

5 Add the APS and TEMED to the Collection gel and pipette it on top of the running gel until the chamber is filled. Quickly add the gel comb to the still-liquid gel and push it to the

desired depth.

6 Let the gel polymerise fully. It can be stored in wet paper in the fridge for a few days until use or used immediately.

Running an SDS gel

7 Prepare your protein solutions by incubating them with 5x Protein loading dye containing SDS for 30 minutes at 50°C to denature the proteins. They can be stored on ice until putting them on the gel or used directly. Use about 20 µg of protein if you intend to dye the gel with 9 Coomassie blue. Use about 3 µg for a western blot.

8 Put the SDS gel into the appropriate chamber and fill the middle of the chamber with cathode buffer and the bottom of the chamber with anode buffer. Gently pull out the comb from the gel.

9 Use a pipette or a needle to straighten out the pockets in the gel and flush them with buffer.

10 Pipette your denatured protein solutions into the pockets.

11 Run the Gel at a constant 45 mA for about 90 minutes 1h 30m

Coomassie Blue Dye

12 Remove the gel from the chamber and the glass and put it into a small tub.

13 Add fixation solution and incubate at 150 rpm for 5 minutes. Recycle fixation solution afterwards.

14 Add Coomassie Blue Dyeing solution and incubate at 150 rpm for 5-10 minutes. Recycle coomassie blue afterwards.

15 Add fixation solution and incubate at 150 rpm for several hours or over night. Pour this solution into the coomassie blue trash afterwards.

16 Add Water to get the gel back into its orignal size, incubate for at least 10 minutes. 1m

17 Image the gel in a GelDoc.Remove the gel from the chamber and the glass and put it into a small tub.