

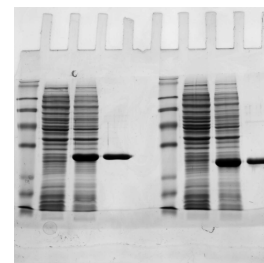
Oct 11, 2022

Version 2

SDS-PAGE V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.5jyl85o7dl2w/v2>



Anna Bird¹, Chiara Gandini¹

¹University of Cambridge

Reclone.org (The Reage...

Open Bioeconomy Lab



Anna Bird

University of Cambridge

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.5jyl85o7dl2w/v2>

Protocol Citation: Anna Bird, Chiara Gandini 2022. SDS-PAGE. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.5jyl85o7dl2w/v2> Version created by **Anna Bird**

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 11, 2022

Last Modified: October 11, 2022

Protocol Integer ID: 71156

Keywords: page sd, page gel, running sd, sd, page, buffer, protein, whole cell sample

Abstract

SDS-PAGE gels are used to visualize proteins. This protocol describes how to prepare all the buffers required for casting and running SDS-PAGE gels, as well as how to prepare whole cell samples.

Protocol materials

 Tris **P212121**

 Glycine

 Sodium Dodecyl Sulfate **P212121**

 Ammonium persulfate **Catalog #A3678**



Buffers

10m

1 4X Resolving Buffer (1.5 M Tris-HCl, pH 8.8)

10m

- Add 90.75 g  Tris **P212121** to  400 mL DI water
- Titrate the solution with ~18% HCl to pH 8.8
- Add water to a final volume of  500 mL
- Store at 4°C

2 4X Stacking Buffer (0.5 M Tris-HCl, pH 6.8)

10m

- Add 30.25 g  Tris **P212121** to  400 mL DI water
- Titrate the solution with ~18% HCl to pH 6.8
- Add water to a final volume of  500 mL DI water
- Store at 4°C

3 10X Running Buffer

15m

- Weigh 30 g Tris  Tris **P212121** .
- Weigh 144 g  Glycine .
- Weigh 10 g  Sodium Dodecyl Sulfate **P212121** SDS.
- Dissolve in  1000 mL water.
- pH should read 8.3. No pH adjustments are needed.
- Dilute to 1X before use.
- Store at room temperature.

4 10% Ammonium Persulfate (w/v)

5m

- Add 1g of  Ammonium persulfate **Catalog #A3678** to  10 mL of DI water
- Store at 4°C

5 10% SDS (w/v)

5m

- Add 10g of  Sodium Dodecyl Sulfate **P212121** to  100 mL DI water
- Store at room temperature



- 6 3X Laemmli Buffer
- 2.4 mL 1 M Tris pH 6.8
 - 3 mL 20% SDS
 - 3 mL glycerol
 - 1.6 mL beta mercaptoethanol
 - a drop of bromophenol blue

10m

Gel Casting

5m

- 7 In an Eppendorf tube combine
- 0.5 mL 30% Acrylamide: Bisacrylamide (29:1)
 - 0.5 mL DI water
 - 10 μ L APS
 - 1 μ L TEMED

Pipette 200 μ L down the right side, and 200 μ L down the left side

Allow to solidify for 00:05:00

8 Resolving Layer

This following recipe makes a 12% SDS-PAGE. For optimal resolution of large proteins (25-200 kDa), you should use smaller concentration of acrylamide (8%), and for resolution of small proteins (4-70 kDa), you should use higher percentage of acrylamide (12-15%). You can calculate a recipe for a different gel percentage using

<https://www.cytographica.com/lab/acryl2.html>

- 8.1 Combine
- 1.645 mL DI water
 - 1.645 mL 30% Acrylamide: Bisacrylamide (29:1)
 - 1.25 mL 4X Resolving Buffer (1.5 M Tris, pH 8.8)
 - 50 μ L 10% SDS
 - 50 μ L 10% APS
 - 5 μ L TEMED

TEMED must be used in a fume hood



Add APS just before casting as the gel begins to polymerize immediately after addition of APS.

- 8.2
- Pour into the mold, leaving ~2 cm below where the bottom of the comb will be
 - Cover with a layer of isopropyl alcohol (IPA)
 - Wait  00:20:00 for gel to solidify

20m

9 Stacking Layer

The stacking layer helps all the proteins get lined up so all proteins enter the resolving layer at the same time

- 9.1 Dump out any excess IPA

10m

Combine

-  2.6 mL DI water
-  1 mL 30% Acrylamide: Bisacrylamide (29:1)
-  1.25 mL Stacking Buffer (0.5 M Tris, pH 6.8)
-  50 μ L 10% SDS
-  50 μ L 10% APS
-  5 μ L TEMED

TEMED must be used in the fume hood

Add APS just before casting as the gel begins to polymerize immediately after addition of APS.

- 9.2
- Pour into the mold
 - Place the comb
 - Wait  00:15:00 for gel to solidify
 - Move to water storage

15m



Sample Preparation

10 Preparation of Whole Cell Samples for SDS-PAGE analysis

Collect whole cell samples before and after induction, and normalize to the cell mass. This allows you to compare the protein expression before and after inducing cells.

- 10.1
- Heat the waterbath or heatblock to 95 °C
 - Read the optical density at 600 nm (OD600) of the cell culture using a photometer.

5m

*Note: the OD600 reading should be within the linear range of the photometer. If the reading appear to be lower than 0.1 or higher than 0.9 concentrate or dilute the cell sample accordingly in order to ensure the OD reading to fall within the 0.1- 0.9 range. If you dilute or concentrate the sample remember to calculate back the initial concentration (e.g. if you diluted 5 times the initial concentration is $y*5$, where y is the reading you have obtained from the diluted concentration)*

- 10.2
- Calculate the amount of culture to harvest to have a cell pellet equivalent to 1mL of OD600 =1.

10m

Calculate the amount of biomass using the formula

$$C_i * V_i = C_f * V_f$$

where C stands for "concentration", V stands for "volume", i stands for "initial" and f stands for "final". Therefore:

$$y \text{ OD600} * x \text{ mL} = 1 \text{ OD600} * 1 \text{ mL}$$

where y is the OD600 reading and x is the volume to be calculated. Therefore

$$x \text{ mL} = (1 * 1) / y$$

- Transfer the amount of culture as calculated into an appropriate tube and centrifuge it at 7,000 rpm for 00:10:00 in tabletop centrifuge
- Discard the supernatant

- 10.3
- Transfer 150 µL of 1X Laemmli Sample Buffer in the tube and resuspend the pellet by pipetting.

11m

- Transfer the tube to the water bath/heatblock, inserting it into the floaters and incubate the tube at 95 °C for 00:05:00
- Transfer the tube on ice for 00:01:00
- Transfer the tube in a bench-top centrifuge and centrifuge at room temperature at max speed (e.g. 13,000 xg) for 00:05:00



- Transfer the supernatant to a final 1.5 microcentrifuge tube. *Note: the pellet won't be visible. Remove the supernatant without touching the bottom of the tube with the pipette tips to avoid carry-over of membranes. This step is necessary to remove membranes and debris that will affect a good quality run of the samples on the SDS-PAGE gel.*
- Load  10 μL of the supernatant on the SDS-PAGE gel.
- Store the remaining sample at  $-20\text{ }^{\circ}\text{C}$.

11 Purified Protein

5m

- Add  2.5 μL of 3X Laemmli Buffer to  7.5 μL of sample
- Incubate  00:05:00 at  $95\text{ }^{\circ}\text{C}$.
- Load the sample onto the SDS-PAGE gel.

Running the Gel

1h 30m

12

- Place in gel running box and cover with running buffer
- If running only one gel, make sure the other side has a dummy gel cassette inserted.
- Fill the space between the two gels with 1X running buffer.
- Add  7.5 μL ladder with dye to a well. Add 7.5 - 10 μL samples to wells.
- Run at 80V until the loading dye reaches the resolving/stacking layer interface.
- Run at 180V until the loading dye reaches the end of the gel.
- Remove gel from casing. Place in petri dish and cover with Coomassie blue dye. Allow to stain overnight.

1h 30m