Aug 01, 2022

Assessing protein purity using SDS PAGE

In 1 collection

Shalo Minette[1], Stephane Fadanka[1], Nadine Mowoh[1]

[1]Beneficial Bio, Mboalab

1 *Works for me* Share

This protocol is published without a DOI.

Nadine Mowoh

ABSTRACT

SDS-PAGE (Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis) is an electrophoresis method that allows protein separation by mass. The gel acts as a sieve through which the proteins move in response to an electric field.

.

SDS-PAGE allows an estimation of the purity of protein samples and can also be used in confirming the presence of a particular protein in a sample through its molecular weight by running it simultaneously on the gel with another protein of known molecular weight, called the standard or protein ladder.

SDS is an anionic detergent and is used to denature the proteins. The negative charges on SDS destroy most of the secondary and tertiary structure of proteins and are strongly attracted toward the anode in an electric field. Because the charge-to-mass ratio is nearly the same among SDS-denatured proteins, the final separation of proteins is almost entirely dependent on the differences in relative molecular weight (MW) of polypeptides.

PROTOCOL CITATION

Shalo Minette, Stephane Fadanka, Nadine Mowoh 2022. Assessing protein purity using SDS PAGE. **protocols.io** https://protocols.io/view/assessing-protein-purity-using-sds-page-cegktbuw

COLLECTIONS

Beneficial Bio: Quality control tests

CREATED

Jul 28, 2022

LAST MODIFIED

Aug 01, 2022

PROTOCOL INTEGER ID

67820

PARENT PROTOCOLS

Part of collection

Beneficial Bio: Quality control tests

GUIDELINES

- This protocol can be used to assess the protein purity by assessing presence of target vs contaminating bands on SDS-PAGE.
- In our lab we typically use this protocol to confirm successful protein expression in preparing cellular reagents.
- In this protocol we use pre-cast TruPAGE gels

MATERIALS TEXT

Reagents

- Loading buffer: SDS Loading buffer
- Sample buffer: TruPAGE LDS Sample Buffer
- Molecular weight marker: BlueRay Pre-stained Protein Ladder (Gene Direx)
- Running buffer (MES/MOPS): Tris-MOPS SDS Express
- Staining reagent: Coomassie Brilliant Blue G-250
- 10% ethanol: Gel De-staining solution
- 20% (w/v) NaCl; Supplement for de-staining solution storage
- Autoclaved distilled water
- DOC lysis buffer (1% w/v Sodium Deoxycholate, 1M Urea, 20mM Tris (pH 8), 10 mM EDTA)

Materials and Equipment

- Crude cell lysate
- Gel tanks: Veri-Gel Complete Mini PAGE system (Fisher Scientific)
- TruPAGE™ Precast Gels: 4-12%, 10 x 10cm, 12-well* 0.1 cm
- P10 or P20 pipette tips.
- Electrophoresis Power-Pack
- Staining dish
- Water bath
- Microwave
- Shaker
- Photometer
- Microcentrifuge
- Pipettes
- Vortex
- Eppendorf tubes

SAFETY WARNINGS

- Wear protective clothing (Lab coat, gloves, face masks)
- Take extra caution if you have to cast your own gels
- Extra care should be employed when handling HCL

BEFORE STARTING

- Ensure all materials to be used for this procedure are all ready and functional

Preparing reagent working stocks

1 Running Buffer Preparation

Before beginning, prepare working stocks of the running buffer, staining solution and lysis buffer.
Also prepare the cell lysate and protein sample for SDS PAGE.

For 1 L of 1x Tris-MOPS buffer

Measure out **50 mL** of 20X Tris-MOPS buffer into a 1L capacity beaker and add **950 mL** of distilled water.

TruPAGE Tris-MOPS SDS Express Running buffers are supplied as 20X concentrated solutions. For best results, it is recommended to prepare a fresh 1X buffer for every run.

2 Staining reagent preparation (Coomassie Brilliant Blue G-250)

- Dissolve **80 mg** of CBB G-250 in **1 L** of bi-distilled water by stirring for 2-4 hours.
- Add **3 mL** of concentrated HCl to the dark blue solution with stirring for another minute and store in the dark for later use.

NB: The solution can be stored for weeks up to several months without losing its staining efficiency.

- Concentrated HCl should be handled with care and used under a fume hood. The final solution will be at about pH **2**, so gloves should be used and any contact with the skin should be avoided.

3 DOC-Lysis Buffer

For 100ml of the buffer;

A	B	C
Component	**Amount required**	**Final concentration in solution**
Sodium deoxycholate	1g	1 %
Urea	6.01g	1M
Tris	2 mL	20 mM
EDTA	2 mL	10 mM

Tris **Roche**

- To prepare 1 L of 1M tris, dissolve **121.4 g** of **Diagnostics Catalog #10056626** in 950 mL of distilled water and add concentrated HCL to adjust the pH to 8.0 and finally add distilled water to adjust the volume to 1 L.

0.5 M EDTA pH 8.0 **Invitrogen - Thermo**

- We use **Fisher Catalog #01105008**

- Measure out the powders into a 250 mL capacity glass beaker as shown in the table above.
- Add the buffers as indicated and finally add **96 mL** of distilled water to completely dissolve the powder.
- The lysis buffer can be stored at **4 °C** and is stable for up to 6 months.

Sample Preparation/Denaturation

4 Preparing whole cell samples

- After protein expression with IPTG or in autoinduction media, read the optical density at 600 nm (OD600) of the cell culture using a photometer.

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

- Calculate the amount of culture to harvest to have a cell pellet equivalent to 1mL of OD600 =1 using the formula:

Ci * Vi = Cf * Vf

where Ci means “initial concentration”, Cf means “final concentration”, Vi means "initial volume", and Vf means "final volume ".

Therefore:(**yOD600) * x mL = 1 OD600* 1 mL**

where Y is the OD600 reading and X is the volume to be calculated.

Therefore, **x mL = (1 * 1)/y (multiply by 1000 µL to convert to ul).**

- Transfer the amount of culture as calculated above in an appropriate tube and centrifuge it at 5,000 xg for 10 minutes.

- Discard the supernatant and resuspend the pellet in Doc lysis buffer (1mL per 0.1g of the pellet).
- Vortex intensively for 2 min at room temperature.
- Leave it shaking on a rotor at room temperature for 15mins.
- Then pellet the cells by centrifugation at 13000 rpm for 5 mins and carefully collect the supernatant which is our cell lysate for SDS PAGE analysis.

5 Preparing the protein samples

- Pre-heat water bath to 95ºC.
- Determine the concentration of the protein sample via Bradford assay.
- Dilute or concentrate samples as needed to yield a final protein concentration of >0.5 mg/mL for purified proteins and <20mg/mL for cell lysates. ***Where this is not applicable, proceed with the next step as indicated.***
- Proceed to mix sample and TruPAGE LDS Sample Buffer as demonstrated in the table below.
- Samples should be prepared just prior to analysis.

Generally, 1.0 µg is sufficient to visualize purified proteins and 10 µg is sufficient to visualize proteins in lysates on a Coomassie stained gel.

For example: Using the formula **CiVi=CfVf**, we can find out what concentration of cell lysate is required to load a sample with a final concentration of 15 ug/uL in a total volume of **50 µL** .
If we use 30 uL of cell lysate;

Ci*30 uL=(15 ug/uL)*(50 uL)
Therefore; Ci= (15 ug/uL)*(50 uL)/30 uL
Ci=25 ug/uL

- So, dilute your sample to an initial concentration of 25 ug/uL before preparation with a sample buffer.

buffer using this protocol.

A	B	C
Reagents	**Amounts (ul)**	**Ex. 50ul reaction**
Protein Sample	x	30
4x sample buffer	12.5	12.5
DTT (1M)	3.5	3.5
Sterilised distilled water	34-x	4
Total volume	50	50

- Place the samples in a water bath at 95ºC for 5 mins.

Setting up the run

6 Setting up the apparatus

- Rinse the gel wells with sterile distilled water. TruPAGE wells MUST be rinsed prior to loading samples for removal of trace storage buffer to achieve optimal band resolution and sharpness.
- Remember to peel off the white tape at the bottom of the gel cassette prior to loading samples.
- Place Precast gel into apparatus and secure as appropriate;
- Fill the cathode chamber with 1X running buffer and ensure a proper seal prior to filling the anode chamber.

7 Loading the wells

- Load the samples into the wells. Do not exceed well capacity when loading samples, 35 uL for 12 well gels and 20 uL for 10 well gels (For TruPAGE gels).

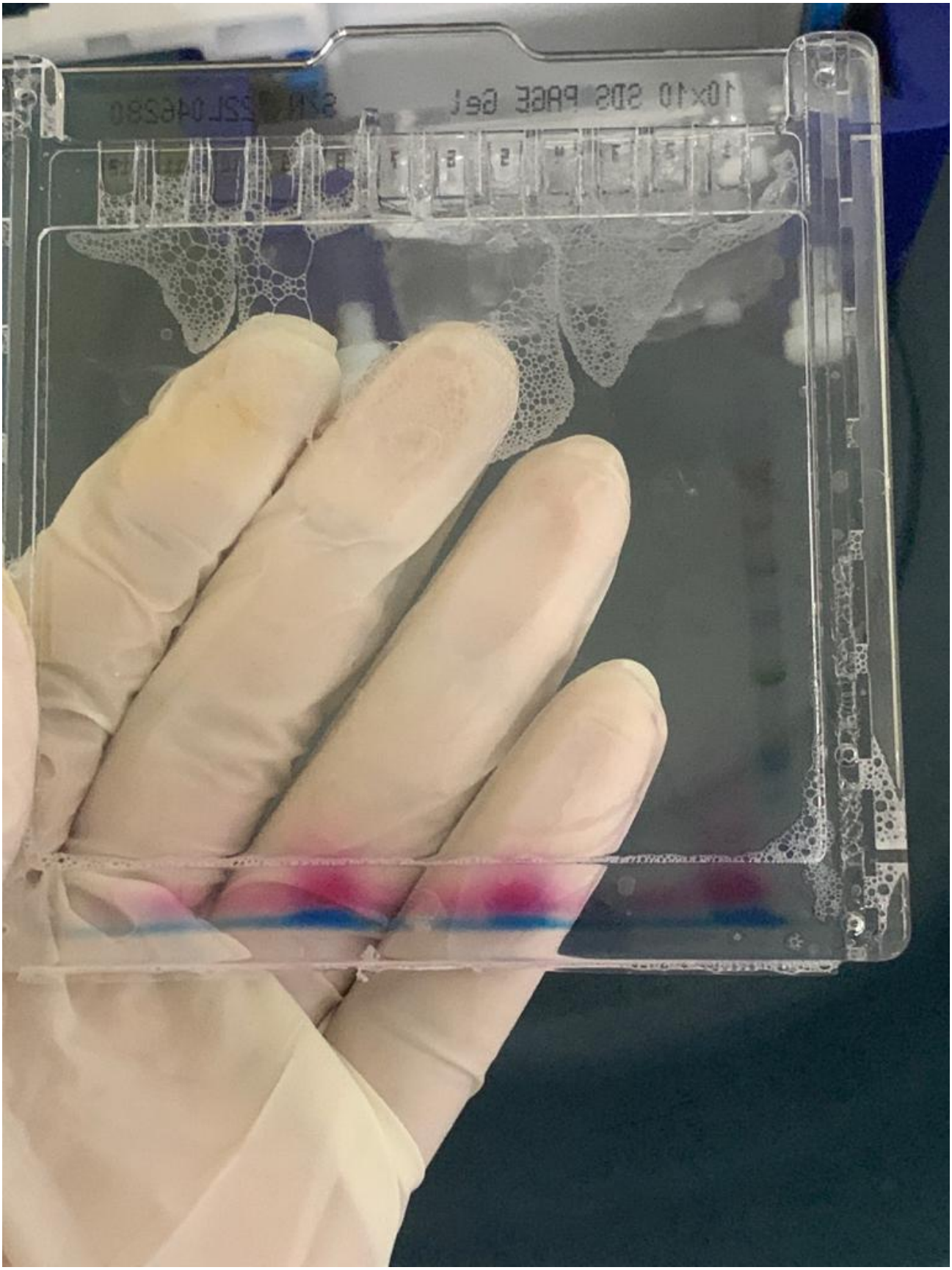
Note: Use thin gel-loading tips for better settling of the samples towards the bottom of the wells.

- Load 10 µl of MW marker in the left most lane.
- Load 10-30 µl of samples depending on well capacity and purity of the protein in subsequent wells**.** Load less for purified proteins (10 uL) and more for cell lysates (20 uL).

8 Running the Gel

- Once the samples are loaded and buffer chambers are filled, cover the chamber and firmly connect both the anode and the cathode.
- Set the voltage on the electrophoresis power supply to a constant voltage of 180 V. Turn ON the power supply.

- Run the gel at constant voltage (180 V) until the blue dye front reaches the bottom of the gel cassette.



- Do not touch the electrophoresis unit while power is on. If the buffer is leaking from the unit be certain to turn power OFF before making contact with the buffer. Use care at all times.
- Depending on the gel percentage and running buffer, run times can range from 30–70 minutes. Expected current during the gel run should remain below 110 mA/gel (maximum: 20 W) and will decrease gradually as the run progresses.

Staining and de-staining the Gel

9

- **This is a heated procedure (50–70 C) and the volumes in this procedure are for 1 gel.**
- **Do not heat the gel to temperatures ABOVE 70 C, as the stain solution may get too hot and dissolve the gel matrix.**

- Prepare Coomassie Brilliant Blue G-250 stain solution as directed above.
- After running the gel, remove the gel from the cassette. This can be tricky, use a spatula to pry open the cassette carefully not to break the gel.
- Heat 100 mL of sterilized autoclaved water for 1 minute on high (600 W).
- Wash the gel with warm ultrapure water for 3 to 5 minutes.
- Repeat warm water wash thrice.

Washing steps are very important for proper visualization of the protein. Residual SDS inhibits protein staging in gels that are not washed long enough.

- Heat 25 mL of stain solution for 20 seconds on high (600 W). Place the gel in the warm stain solution and incubate for 30 minutes with gentle agitation.
- Pour off the staining solution and add 50-100ml bi distilled water in order to de-stain the light blue background on the gel. Place on a shaker and replace water if necessary for further de-staining.
- If the gel is stained for more than 30mins, warm 100 mL of de-stain solution (10% ethanol) for 30 seconds on high (600 W). Remove gel from stain solution and de-stain for 15 minutes to 16 hours. *The duration of de-staining depends on the gel type used.*
- Document the gel (take a clear picture).