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SPS-PAGE protein electrophoresis

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We use this protocol and it's working

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Abstract

SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) is a variant of PAGE that allows to separate protein molecules according to their molecular masses under electric field.

SDS-PAGE is a discontinuous electrophoretic system developed by Ulrich K. Laemmli which is commonly used as a method to separate proteins with molecular masses between 5 and 250 KDa.

In this protocol we will use an already precasted gel.

Guidelines

In this protocol we use a precasted gel.

Materials

Material/Consumables:

- 1x TGS Buffer
- Nuclease free water
- 4-15% Precast Polyacrylamide gel in glass frames
- Long pipette tips
- Prestained Protein ladder
- Precision wipes
- Distilled water
- Bio-safe Coomassie stain

Equipment:

- Vertical electrophoresis system
- Buffer dam
- Power supply unit
- Heating element (Thermal Cycler, Eppendorf ThemoMixer, etc)
- Shaker

Before start

For a proper experiment you will need at least one of the wells for a protein ladder and one for negative control. In the rest of the wells you will load 10µl of sample, so depending on the nature of your experiment and the number of wells in your gel, you can calculate how many different samples and/or replicates can be tested during a single run.

Sample preparation

1 In a tube add 10µl of 2x Laemmli Buffer , 5µl Nuclease free water and 5µl of your protein sample.

Note

You may adjust the ratio of water:protein depending on the concentration of proteins in your sample.
e.g. for high concentration add 7µl of water and 3µl of proteins
for low concentration add 3µl of water and 7µl of proteins

1.1 Mix 10µl of 2x Laemmli Buffer with 10µl Nuclease free water to use as a negative control.

2 Mix well, either pipetting up and down, vortex or flicking the tube. Afterwards, a few seconds of centrifuge might be needed to collect all the sample back at the bottom of the tube.

3 Incubate the tube at 100^{o}C for 15 minutes in a dry heating element.

Note

For PCR tubes you may use a Thermal Cycler. Do not forget to set Heated Cover Temperature to 105^{o}C to avoid condensation.

Note

After 15min you can store the samples at 4^{o}C until you start the electrophoresis

Electrophoresis system preparation

4 Rinse the tank, the buffer dam (if you want to run an odd number of gels) and the casting stand.

5 Open the casting gel, remove the comb and the plastic cover on the bottom and rinse well.

6 Insert the gel(s) in the casting stand(s). If you want to run one or three gels you must include a buffer dam to complete the set.

Note

Add some water inside the casting stand between the two gels. If there is no leakage then the gels are mounted properly. Discard the water.

7 Put the casting stand in the tank and fill the inner compartment with 1x TGS Buffer. Then fill the tank until the appropriate marked line.

Note

For one or two gels until the 2 Gels mark, for three or four gels until the 4 Gels mark

Loading the samples

8 It is better to use long tips. 20µl tips can also be used but it requires more effort to load the samples without damaging the gel.

9 For the ladder load 3µl in one well, while for the samples and the negative control load 10µl per well.

Note

To make sure that the tip is actually inside the well move it back and front. If your movement is not constricted by the glass frames this means that you are not in the well. Pipette slowly to make sure that no sample will be propelled outside the well.

SDS-PAGE electrophoresis

10 Close the lid and connect the electrodes on the power supply.

Note

Make sure that the electrodes are attached to the right position (red in red and black in black)

11 Set the power supply to 120Volts for 5 minutes.

Note

After a while you should be able to see some bubbles, if not, then there is a problem with the circuit. Check the cables and add some more buffer in the tank and between the gels

11.1 Then set the power supply to 200Volts and run for 25 minutes

Note

By the end of the 25 minutes the blue band that appears on the gels should be close to the bottom.
You may run the gel for a couple more minutes at 200volts so the band will be even closer to the end (to achieve better separation) but be careful not to surpass it.

Gel staining

12 Take the casting stand of the tank and pour the buffer in a bottle along with the buffer inside the tank. It can be used for multiple gel runs.

13

Note

Every time you touch the gel use clean gloves to avoid contamination.

Carefully open the glass frames. Use the specialized tool or a metal object that can fit in the frame openings.

Note

Leave the gel attached to one of the glass frames so you can transfer it easily.

14 Clean the gel.

14.1 Pour distilled water in a bowl.

Note

Keep in mind that in a following step we will put the bowl in a shaker, so be careful not to overflow it.

Then put the gel in the bowl.

Note

You can flip the frame in the water an shake gently. Water will come between the gel and the frame so it will be released without any damages

14.2 Put it on a shaker for 5 minutes.

14.3 Remove the water, then add some more clean one and shake for another 5 minutes

Note

Press the gel to the bottom of the bowl using your fingers to avoid risking it falling in the sink

14.4 Repeat step 14.3 one more time

15 Remove the water from the bowl and add around 50ml of Bio-Safe Coomassie Stain

Note

Depending on the size of your bowl , you might need some more stain so the gel is completely covered.

16 Put on the shaker for one hour.

Gel Washing

17 Remove the Coomassie stain and rinse the gel very well

18 Add some distilled water on the shaker and let it overnight (12-16 hours)

Note

You may add some precision wipes in the bowl that will absorb some of the color.
Try not to put them on top of the gel because they might be attached to it and when you remove them it will damage the gel

18.1 Optionally after some hours you can renew the water in the bowl and change the wipes.

Note

Do not overexpose the gel to water because after some time the desired bands might also start to fade away.

Visualization of results

19 Carefully remove the gel from the bowl and put it in a transparent colorless plastic file folder.

20 The image of the gel can be captured using a conventional scanner or with the white light mode on a UV transilluminator