STANDARD OPERATING PROCEDURE

Title: Evaluation using Sodium Dodecyl Sulfate (SDS) PolyAcrylamide Gel Electrophoresis (PAGE)

SOP#: M-100

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1. PURPOSE

The purpose of this document is to describe the procedure for performing Sodium Dodecyl Sulfate (SDS) PolyAcrylamide Gel Electrophoresis (PAGE) for the separation and characterization of proteins.

2. SCOPE

This procedure may be used for proteins with molecular weights in the range of 10KDa to 250KDa. Other procedures must be used for proteins outside of this range.

3. RESPONSIBILITIES

It is the responsibility of person(s) performing this procedure to be familiar with lab safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

4. EQUIPMENT

- BioRad Criterion® Cell apparatus or equivalent
- BioRad PowerPac HC power supply or equivalent
- Water bath or heating block pre-heated to 90°C
- Rocking platform; Labline Model 4831 or equivalent
- Epson photo quality scanner or equivalent
- Precision pipettes

5. MATERIALS

- Tris/glycine SDS Buffer (prepared); BioRad Cat. #161-0732
- Polyacrylamide gel (12 or 18 lane); BioRad Cat. #345-0028

- Laemmli Sample Buffer; BioRad Cat. #161-0737
- Phosphate Buffered Saline (PBS), 10x solution (Fisher Scientific Cat. #BP399-1) diluted to 1x with deionized water to give 11.9 mM phosphate, 137mM NaCl, 2.7mM KCl, pH 7.4
- Dual Color Standard; BioRad Cat. #161-0374
- Coomassie Briliant Staining Solution; BioRad Cat. # 161-0436
- Destain Solution; BioRad Cat. # 161-0438

6. REAGENTS

- Sample Antigen to be tested

7. PROCEDURE

7.1. Make 1mg/ml Stock Solution of Antigen (Ag) in PBS

7.1.1. Determine the concentration of the protein by either BioRad Protein Assay (SOP; M-101), OD280 using Nanodrop (SOP; I-101) or by the concentration listed on the label.

7.1.2. Dilute the sample antigen to a 1mg/ml stock solution in a final volume between 50 and 100 µl.

7.2. Prepare SDS/Ag Solution (1:4 Dilution)

7.2.1. Pipet 60 µl of Laemmli Sample Buffer into 1 ml Vial

7.2.1.1. Laemmli Sample Buffer contains dye and SDS

7.2.2. Pipet 20 µl of Ag (1 mg/ml) into Laemmli Sample Buffer (6.2.1)

7.2.2.1. Final SDS/Ag Solution concentration is 0.25 mg/ml

7.3. Denature SDS/Ag Solution

7.3.1. Heat SDS/Ag Solution @ 90°C for 5 minutes in a pre-heated water bath or heating block. This will denature the protein and will give the protein a negative charge.

7.4. Load SDS-PAGE

7.4.1. Add 1x Tris/glycine SDS Buffer to the fill line on the electrophoresis chamber

7.4.2. Insert pre-made polyacrylamide gel into the chamber

7.4.3. For 12 lane gel:

7.4.3.1. Pipet 10 µl of Dual Color Standard into lanes 1, 5 and 9

7.4.3.2. Pipet 10 µl of SDS/Ag solution to be tested into lanes 2, 3, 4, 6, 7, 8, 10, 11 and 12

7.4.4. For 18 lane gel:

7.4.4.1. Pipet 10 µl of Dual Color Standard into lanes 1, 5, 9 and 13

7.4.4.2. Pipet 10 µl of SDS/Ag solution to be tested into lanes 2, 3, 4, 6, 7, 8, 10, 11, 12, 14, 15 and 16

7.5. Run SDS-PAGE

7.5.1. Connect the Criterion Cell chamber to the power supply and set the voltage to 200 volts.

7.5.2. Let the gel run until the low molecular weight proteins are near the bottom of the gel, as evidenced by the pre-stain marker and/or the dye front. This will take ~ 1hour.

7.5.3. Check the gel regularly to determine how far the proteins have migrated.

7.5.4. Stop the procedure when the blue dye front is within 1 cm of the bottom of the gel.

7.5.5. Gently remove the gel and rinse the gel and chamber with deionized water.

7.5.6. Use cassette opening tool on top of chamber (as described in Criterion Cell Instruction manual (165-6001; page 5, section 2.3).

7.5.7. Pry open the cassette carefully and make sure the part with the lip is face down (this is where you want your gel to end up).

7.6. Coomassie Stain/Destain of SDS-PAGE

7.6.1. Carefully transfer gel to a plastic 6 x 6 inch container.

7.6.2. Fill container with Coomassie Stain so that the gel is completely submerged.

7.6.3. Stain for 1 hour at room temperature with gentle rocking.

7.6.4. Remove the Coomassie Stain by carefully decanting ensuring that the gel remains in the bottom of the container

7.6.5. Fill container with Destain Solution so that the gel is completely submerged.

7.6.5.1. Destain for 30 minutes at room temperature with gentle rocking.

7.6.5.2. Remove the Destain Solution by carefully decanting.

7.6.5.3. Repeat the destain process until gel background is clear.

7.6.5.4. Once gel background is clear, remove the Destain Solution by carefully decanting. Cover the gel with deionized water.

7.7. Scan SDS-PAGE

7.7.1. The final gel image should be scanned using a photo quality scanner (i.e. Epson or equivalent).

7.7.2. Place the gel carefully on the scanner. Remove any bubbles with the BioRad roller apparatus.

7.7.3. Cover with a plastic sheet protector containing a piece of plain white paper.

7.7.4. Carefully close the scanner and scan at the highest possible resolution. Store the image as a JPEG or TIFF file.

7.7.5. Annotate as appropriate in Adobe Photoshop.

8. REFERENCED DOCUMENTS

8.1. Hames, B.D. and Rickwood, D. (eds.) 1990.Gel Electrophoresis of Proteins: A Practical Approach, 2nd ed. Oxford University Press, New York.

8.2. http://www.jove.com/index/details.stp?ID=758

8.3. Gallagher, S. Electrophoretic separation of proteins. Current Protocols in Molecular Biology, (2006) Supplement 75, 10.2.1

8.4. SOP M-101: Protein Deterination by BioRad Protein Assay.

8.5. SOP I-101: Protein determination by OD measurement using the NanoDrop Instrument.