Mount Wachusett Community College
27 Jackson Rd.
Devens, MA 01434

Document Number:1.23
Revision Number: 2
Effective Date: 13Jun18

SOP: SDS-PAGE

Approvals

Preparer:	Lara Dowland	**Date:**	19Jan10
Reviewer:	Cianna Cooper	**Date:**	13Jun18

1. **Purpose:**
   1.1. To describe the appropriate operating instructions to perform SDS PAGE analysis of proteins samples.
2. **Scope:**
   2.1. Applies to confirming the presence and purity of protein samples.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Invitrogen Novex Gel instructions
   4.2. Fisher Scientific Vertical MiniGel System SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Acrylamide is a neurotoxin. Always wear protective gloves when handling the polyacrylamide gels.
7. **Materials:**
   7.1. Protein Samples
   7.2. Precision Plus Protein Standards, Kaleidoscope from BioRad (2– 8°C refrigerator)
   7.3. Fisher Scientific Vertical MiniGel System
   7.4. Power Supply for Protein Electrophoresis
   7.5. NuPAGE 4-12% Bis-Tris Gels (1.0mm x 10 well), 2– 8°C refrigerator
   7.6. NuPAGE MOPS SDS Running Buffer (20X), room temperature
   7.7. NuPAGE Antioxidant, 2– 8°C refrigerator
   7.8. NuPAGE LDS Sample Buffer (4X), room temperature
   7.9. Reducing Agent (10X), -20°C freezer
   7.10. Graduated cylinders (1L and 100ml)
   7.11. P20, P100 or P200 Micropipettor and tips, including gel loading tips
   7.12. Microfuge Tubes
   7.13. Microfuge
   7.14. Heat block
   7.15. Staining Trays
   7.16. rotary shaker
   7.17. Invitrogen Simply Blue Safe Stain
   7.18. Light Box
   7.19. Gel Documentation Instrument
8. **Procedure:**

SOP: SDS-PAGE

8.1. **Prepare Running Buffer and Staining Solutions**

8.1.1. 1L NuPAGE MOPS SDS Running Buffer (1X) (if needed)

8.1.1.1. Place 50ml NuPAGE MOPS SDS Running Buffer (20X) in a 1 Liter graduated cylinder.

8.1.1.2. Gently add 950ml deionized water by running it down the side of the cylinder to make 1 liter of 1X NuPAGE MOPS SDS Running Buffer.

8.1.1.3. Add a stir bar and gently stir. NOTE: SDS is a detergent and will foam if mixed vigorously.

8.1.2. 200ml NuPAGE MOPS SDS Running Buffer (1X) plus antioxidant (if needed)

8.1.2.1. Add 200ml of 1X NuPAGE MOPS SDS Running Buffer into a 500ml Erlenmeyer flask.

8.1.2.2. Add 500µl of NuPAGE Antioxidant.

8.1.2.3. Add a stir bar and gently stir. NOTE: SDS is a detergent and will foam if mixed vigorously.

8.1.2.4.Store at 2– 8°C

8.2. **Prepare Protein Samples**

(Do NOT perform this step with the Molecular Weight Marker)

8.2.1. Dilute conditioned media from cell culture or flow through fraction off the chromatography column 1:4 in 1x PBS as follows:

8.2.1.1. Pipette 9 ul of 1x PBS into 1.5ml centrifuge tube.

8.2.1.2. Add 3 ul of sample to the same tube.

8.2.2. For **all** the samples, combine the following in a sterile 1.5ml microfuge tube:

2.5ul 4x sample buffer
1.0ul 10x reducing agent
5ul sample
1.5ul deionized water

8.2.3. Mix gently with a pipet by aspirating and dispensing at least 3 times

8.2.4. Heat at 70°C for 10 minutes.

8.2.5. Pulse all samples and standards in a microfuge for 30 seconds.

8.3. **Prepare Fisher Scientific Vertical MiniGel Gel Box**

8.3.1. Assemble gel box according to the Fisher Scientific Vertical MiniGel System SOP.

8.3.2. Place 200ml NuPAGE MOPS SDS Running Buffer (1X) plus antioxidant in upper buffer chamber (small chamber between 2 gels or the gel and buffer dam)

8.3.3. Fill the lower buffer chamber with approximately 600ml of 1X NuPAGE MOPS SDS Running Buffer (large chamber).

8.3.4. Rinse gel wells with micropipettor and buffer from upper buffer chamber.

8.4. **Load Samples**

8.4.1. Using a micropipettor and gel loading tips, load 10ul of the Molecular Weight Marker into one well and 10µl of each sample into separate wells.

8.4.1.1. Avoid loading samples symmetrically.

8.4.2. Load any empty wells with 15µl of diluted 4X LDS Sample Buffer.

8.4.3. Record order of samples and volumes loaded.

SOP: SDS-PAGE

8.5. **Run NOVEX NuPAGE MOPS SDS Precast Gel**

8.5.1. Plug electrophoresis chamber into the gel electrophoresis power supply.

8.5.2. Run gel at 125V for 1 hour.

8.5.3. Turn off the power supply when the dye reaches 1cm from the bottom of the gel.

8.6. **Stain and Photodocument the NOVEX NuPAGE MOPS SDS Precast Gel**

8.6.1. Disassemble gel box per Fisher Scientific Vertical MiniGel System SOP and remove gel from plastic cassette.

8.6.2. Rinse gel box well with DI water. Do not use brushes on the gel box, they scratch the surface. Do not immerse top of gel box or electrical components.

8.6.3. Place gel in staining tray with 100ml of deionized water for 5 minutes with gentle shaking.

8.6.4. Repeat wash step 2x for a total of 3 washes.

8.6.5. Add 20ml of SimplyBlue SafeStain, cover with saran wrap, and gently shake for 1 hour at room temperature.

8.6.6. Wash in 100ml of deionized water for 1 hour or overnight with gentle shaking.

8.6.7. Remove gel from staining tray and place on visible light box

8.6.8. Identify the protein standards and samples and estimate their molecular weights. See Molecular Weight Diagram.

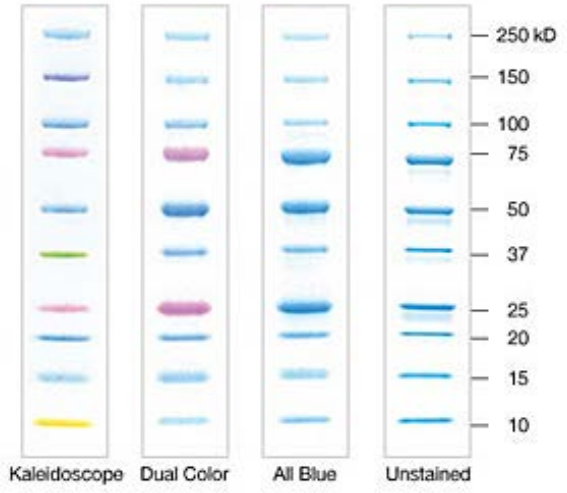
9. Attachments:

9.1. Molecular Weight Marker Diagram

10. History:

Name	Date	Amendment
Lara Dowland	19Jan10	Initial Release
Lara Dowland	13Jun18	Update running conditions and staining procedure.

SOP: SDS-PAGE


250 kD
150
100
75
50
37
25
20
15
10
Kaleidoscope
Dual Color
All Blue
Unstained