Title: SDS-PAGE SOP

Approvals:
Preparer:_____Deb Audino_____Date ____03Apr08_____
Reviewer:_____Kari Britt_____Date ____03Apr08_____

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 the two human proteins (tPA and HSA) we have produced and purified in this class.
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. Novex XCell II Mini-Cell Gel Box Operation SOP
   4.3. gel documentation instrument SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Acrylamide is a neurotoxin. Always wear protective gloves when handling the polyacrylamide gels.
   6.2. Fixative Solution is acidic and flammable. Keep it away from sparks and flames. Dispose in Fixative Hazardous Waste bottle
   6.3. GelCode Blue is harmful. Dispose in GelCode Blue Harzardous Waste bottle.
7. **Materials:**
   7.1. protein samples
   7.2. protein standard, 4mg/mL
   7.3. molecular weight marker (SeeBlue® Plus 2 Pre-stained Standard by Invitrogen is recommended. Catalog number: LC 5925)
   7.4. NOVEX Precast Gel Box and accessories
   7.5. power supply for protein electrophoresis
   7.6. NuPAGE 4-12% Bis-Tris Gels (1.0mm x 10 well)
   7.7. NuPAGE MOPS SDS Running Buffer (20X)
   7.8. NuPAGE Antioxidant
   7.9. NuPAGE SDS Sample Buffer (4X)
   7.10. reducing agent (10X)
   7.11. graduated cylinders (100mL, 250mL, 1L)
   7.12. P20, P100 or P200 Micropipettor and tips, including gel loading tips
   7.13. microfuge Tubes
   7.14. microfuge
   7.15. boiling water bath
   7.16. staining trays
   7.17. rotary shaker

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7.18. Fixative Solution
7.19. Pierce GelCode Blue Staining Reagent
7.20. light box
7.21. gel documentation instrument

8. Procedure:

8.1. **Prepare Running Buffers and Fixative Solution if needed.**

8.1.1. **Lower Buffer:** 1X NuPAGE MOPS SDS Running Buffer (1Liter)

8.1.1.1. Place 50mL of 20X NuPAGE MOPS SDS Running Buffer 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. **Upper Buffer:** 1X NuPAGE MOPS SDS Running Buffer plus antioxidant (200mL)

8.1.2.1. Add 200mL of 1X NuPAGE MOPS SDS Running Buffer to an appropriate vessel.

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.

8.1.3. **Fixative Solution** (500mL)

8.1.3.1. In a 500mL bottle, mix together:
250mL 100% Methanol
215mL deionized water
35mL glacial acetic acid

8.1.3.2. Store at 2– 8°C until needed.

8.2. **Prepare Protein Samples.**

Note: Do NOT perform this step with the Molecular Weight Marker.

8.2.1. For all the samples and the standards, combine the following in a sterile microfuge tube:
25µL 4x sample buffer
10µL 10x reducing agent
65µL sample

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

8.2.3. Boil for 3-5 minutes.

8.2.4. Remove from boiling water bath.

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

8.3. **Prepare Novex Precast Gel Box.**

8.3.1. Assemble gel box according to its SOP.

8.3.2. Place 200mL NuPAGE MOPS SDS Running Buffer (1X) plus antioxidant in the 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).

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8.4. **Load Samples.**

8.4.1. Using a micropipettor and disposable tips, load 10µL of the Molecular Weight Marker into one well and up to 50µL 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 Sample Buffer.

8.4.3. Record order of samples and volumes loaded.

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

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

8.5.2. Run gel at 200V for 40 – 60 minutes.

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 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.

8.6.4. Wash gel 3 times for approx. 5 minutes with DI water shaking at room temp.

8.6.5. Add enough Fixative solution to completely cover the gel and fix for approx. 15 minutes shaking at room temp.

8.6.6. Discard Fixative Solution into the Fixative Hazardous Waste bottle

8.6.7. Wash gel 3 times for a minimum of 5 minutes with DI water shaking at room temp.

8.6.8. Add about 50mL of GelCode Blue and stain for 1-24 hours shaking at room temp.

8.6.9. Decant GelCode Blue into GelCode Blue Hazardous Waste bottle.

8.6.10. Wash gel with DI water for 15 minutes to overnight on a shaker

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

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

9. Attachments:

9.1. Figure 1: Molecular Weight Marker Diagram

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
SCP	2003	Changed Coomassie stain to GelCode Blue Stain
Deb Audino	2005	Put into SOP 2005 format
Deb Audino	09May06	Removed dilute protein standards
Deb Audino	04Apr08	College name change

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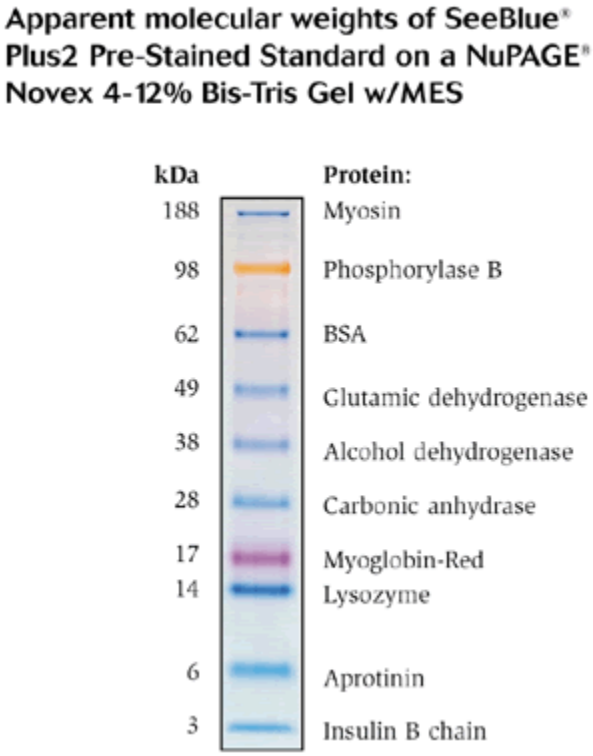

Figure 1: Molecular Weight Marker Diagram