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1.0 Purpose

This method is a staining protocol to provide detection of proteins separated by polyacrylamide gels using Sypro Ruby Protein stain. Sypro Ruby delivers a linear quantitation range over three orders of magnitude, and shows little protein-to-protein variability compared to Coomassie Blue and Silver Stain.

2.0 Scope

Process Analytics (PA) personnel will perform this procedure. Other Development Program (BDP) personnel may use this protocol for development or in-process analysis.

3.0 Authority and Responsibility

3.1 The Director of Process Analytics (PA) has the authority to define this procedure.

3.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA personnel are responsible for performing this procedure.

3.4 PA is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

4.1 Supplies

4.1.1 Sypro Ruby Stains Protein Gel Stain (Molecular Probes, Eugene, OR 514-465-8300, BDP PN 30254, www.probes.com.

4.1.2 Appropriate staining gel trays specific to Sypro Ruby Stain (polypropylene dishes, such as Rubbermaid, avoid glass trays, etc.)

4.1.3 Aluminum Foil, (BDP PN 20012).

4.1.4 Shaker or rocker for agitation during incubations.

4.1.5 Milli-Q Water, Direct-Q Water, HPLC-grade, or equivalent.

4.1.6 Methanol (BDP PN 30853).

4.1.7 Acetic Acid (BDP PN 30860).

4.1.8 Gel Dry Drying Solution (BDP PN 30041).

4.1.9 Trichloroacetic acid (TCA), (BDP PN 30252).

4.1.10 Ethyl Alcohol, 200 Proof, USP, (BDP PN 10106).

4.2 Staining Solutions

Prepare each of the following solutions.

NOTE: All solutions should be given a PA number and recorded in the Solutions logbook per **SOP 22702*, Solutions Used in Process Analytics***. Label all solutions with the PA number, date, preparer's initials, and the expiration date. The final volumes of the solutions listed below may be adjusted as needed as long as the following ratios are used.

4.2.1 Fixing/Destain Solution:

1L prepared as: 300 mL methanol, 100 mL acetic acid, 600 mL Direct-Q water, store at room temperature.

4.2.2 Fixative Solution

1L prepared as: 400 mL methanol, 100 mL trichloroacetic acid, 500 mL Direct-Q water, store at room temperature. Solution can be used for ≤ 6 months.

4.2.3 Sypro Ruby Protein Staining Solution

Stable for at least 6 months when stored at room temperature, protected from light in a container wrapped in aluminum foil.

4.2.4 Washing Solution

1L prepared as: 100 mL methanol (or ethanol), 70 mL Acetic acid, 830 mL Direct-Q water, store at room temperature.

NOTE: Wear gloves both to protect the operator from the chemicals used and to protect the gel from proteins on fingers.

The hazards of Sypro Ruby stain have not been thoroughly investigated. Handle all chemicals with caution. Prepare in a chemical fume hood.

5.0 Protocol Using Sypro Ruby for NuPAGE 4-12% Bis-Tris Gels

5.1 After running NuPAGE 4-12% Bis-Tris gels (**SOP 22176, *SDS Page Gel Electrophoresis Using the NuPAGE Bis-TRIS Gels***), place the gel in Destain Solution and allow the gel to incubate for at least 10 minutes at room temperature. This solution fixes the proteins in the gel.

5.2 Remove the solution and rinse the gel 2 times with high purity water for 10 minutes each time.

5.3 Remove the solution, add 200 mL of Sypro Ruby Staining Solution for a 1.0 mm gel thickness and allow the gel to stain overnight at room temperature with agitation.

NOTE: It is imperative that the gel stain overnight in the Sypro Ruby stain to allow the stain to completely penetrate the protein bands. Sypro Ruby is light sensitive so the container needs to be wrapped in aluminum foil.

- Do not dilute the stain; diluted stain will result in decreased sensitivity.
- Do not reuse the staining solution; this will result in a significant loss of sensitivity.

5.4 Remove the Sypro Ruby stain solution and dispose of as a hazardous chemical liquid waste. Add 200 mL of the Washing Solution and incubate for 30 minutes at room temperature with agitation (keep the container wrapped with foil). Decant the Washing Solution and repeat with 200 mL of Washing Solution for another 30 minutes.

5.5 Scan gel within 1-2 hours of washing using ChemiDoc MP imaging Systems, refer to **SOP 23132, *ChemiDoc MP imaging with Image Lab Software BIO-RAD.***

NOTE: If gel is left longer than 2 hours in the washing solution, gel can be restained.

5.6 Place the gel into Gel Dry Drying Solution for permanent storage. Make sure the gel is submerged in the Gel Dry Drying solution, as per **SOP 22161, *Invitrogen Gel Dry***.

5.7 As per the requestor, the gel can be kept in Sypro Ruby or restained in Coomassie Blue (**SOP 22175, *Staining of Gels with Coomassie Blue R-250*)** or Silver Stain, beginning with step 6.2 – Sensitizer (**SOP 22177, *Silver Xpress Silver Staining*)**.

5.8 Once gel is restained with Coomassie Blue or Silver Stain, place the gel into the Gel Dry Drying Solution for permanent storage. Make sure the gel is submerged in the Gel Dry Drying Solution.

NOTE: Proteins present at very low levels may no longer be detectable after gel drying.

6.0 Protocol Using Sypro Ruby for IEF Gels

6.1 After running IEF gels, **SOP 22181, *Isoelectric Focusing***, place the gel in fixative solution for 30-60 minutes.

6.2 Dispose of the fixing solution in a liquid chemical hazardous waste container, and wash the gel three times with high purity water for 10 minutes each time.

6.3 Dispose of water, add Sypro Ruby Staining Solution (200 mL for a 1.0 mm gel size), and allow the gel to stain overnight at room temperature with agitation.

NOTE: It is imperative that the gel stain overnight in the Sypro Ruby stain to allow the stain to completely penetrate the protein bands. Sypro Ruby is light sensitive so the container needs to be wrapped in aluminum foil.

- Do not dilute the stain; diluted stain will result in decreased sensitivity.
- Do not reuse the staining solution; this will result in a significant loss of sensitivity.

6.4 Remove the Sypro Ruby stain solution and dispose of as a hazardous chemical liquid waste. Add 200 mL of Washing Solution and incubate for 30 minutes at room temperature with agitation (keep the container wrapped with foil). Decant the Washing Solution and repeat - adding an additional 200 mL of Washing Solution and incubating for 30 minutes.

6.5 Scan the gel within 1-2 hours of washing using ChemiDoc MP imaging Systems, refer to **SOP 23132, *ChemiDoc MP imaging with Image Lab Software BIO-RAD***.

NOTE: If a gel is left longer than 2 hours in washing solution, it can be restained.

6.6 Place the gel into Gel Dry Drying Solution for permanent storage. Make sure the gel is submerged in the Gel Dry Drying solution, as per **SOP 22161, *Invitrogen Gel Drying***.

6.7 As per the requestor, the gel can be kept in Sypro Ruby or restained in Coomassie Blue (**SOP 22175, *Staining of Gels with Coomassie Blue R-250*)** or Silver Stain (**SOP 22177, *Silver Xpress Silver Staining***).

NOTE: Proteins present at very low levels may no longer be detectable after gel drying.

7.0 Interpretation of Scanned Gels

7.1 Personnel may use Gel-Pro Analyzer software for densitometry see **SOP 22906, *Operation of the Gel-Pro 6.0 Analyzer Software for Densitometry***

7.2 Personnel may use Molecular dynamics software or the appropriate software.

8.0 References and Related Documents

SOP 23132 ChemiDoc MP Imaging systems with Image Lab Touch Software

SOP 22161 *Invitrogen Gel Drying*

SOP 22175 *Staining of Gels with Coomassie Blue R-250*

SOP 22176 *SDS Page Gel Electrophoresis Using the NuPAGE Bis-TRIS Gels*

SOP 22177 *SilverXpress Silver Staining*

SOP 22181 *Isoelectric Focusing*

SOP 22702 *Solutions Used in Process Analytics*

SOP 22906 *Operation of the Gel-Pro 6.0 Analyzer Software for Densitometry*

Electrophoresis Chapter 10 in Current Protocols in Protein Science, 1995.

Molecular Probes Product Information for Sypro Ruby Protein Gel Stain.

Pharmacia Fluorescence Imaging Manual.

9.0 Change Summary