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1.0 Purpose

This method is a staining procedure to provide detection of proteins in polyacrylamide gels using Coomassie Brilliant Blue R-250 dye. Coomassie blue dye binds nonspecifically to almost all proteins, which allows detection and quantitation of proteins in polyacrylamide gels.

2.0 Scope

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

3.0 Authority and Responsibility

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

3.2 PA personnel are responsible for performing this procedure.

3.3 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this operation.

4.0 Materials and Equipment

4.1 Supplies

4.1.1 Appropriate staining trays (glass, polypropylene, stainless steel, etc.)

4.1.2 Shaker or rocker for agitation during incubations.

4.1.3 High quality water – Milli-Q H_2O, Direct-Q H_2O or WFI.

4.1.4 Coomassie Brilliant Blue R-250 (BDP PN 30250), Brilliant Blue R concentrate (BDP PN 30610, or BDP approved equivalent).

4.2 Preparation of Staining Solutions

4.2.1 Fixative/Destain Solution:

(30% methanol/10% acetic acid) for 1 L measure 300 mL methanol, 600 mL water, and 100 mL acetic acid. Mix well and store at room temperature.

Record in the Solution Logbook, as per **SOP 22702, *Solutions Used in BQC***.

4.2.2 Coomassie Brilliant Blue R-250 Staining Solution (BDP PN 30250):

Mix 2.5 g Coomassie Brilliant Blue R-250, 460 mL of methanol and 460 mL of water. Stir at room temperature. Add 80 mL of glacial acetic acid and continue stirring until mixed. Store solution at room temperature. This solution can be reused several times as long as the solution is filtered if particles appear.

4.2.3 Brilliant Blue R Concentrate:

Pour the contents of the bottle (473 mL) into a 1L graduated cylinder and add 527 mL of water. Stir at room temperature. Store at room temperature

Record in the Solution Logbook as per **SOP 22702, *Solutions Used in BQC***.

NOTE: Gloves must be worn both to protect the operator from the chemicals used and to protect the gel from proteins on fingers.

5.0 Procedure for Using Coomassie R-250 for SDS-PAGE Gels or NuPAGE 4-12% Bis/Tris Gels

5.1 After running the SDS-PAGE Gel or NuPAGE Gel (**SOP 22101*, SDS-PAGE Gel Electrophoresis Using Tris-Glycine Gels,*** *or* **SOP 22176*, SDS Page Gel Electrophoresis Using the NuPAGE® Bis-TRIS Gels*** respectively) the gel is rinsed with Fixative/Destain Solution and incubated in fresh fixative solution for at least 5 minutes.

5.2 Remove the fixative solution, add Coomassie Blue Staining Solution, or Coomassie Brilliant Blue R-concentrate solution, and allow the gel to stain for at least 3 hours with gentle agitation on the shaker.

5.3 Remove the stain solution, add the Fixative/Destain Solution and incubate with agitation in order to destain the gel. This solution can be replaced with fresh solution to promote destaining.

5.4 Continue replacing with fresh solution and incubating as many times as necessary to remove all unbound Coomassie blue dye from the gel. Do not allow the gel to remain in destain for extremely long periods as it will eventually extract dye from the protein bands. If this does occur, the gel will have to be restained.

5.5 When the gel is sufficiently destained, the gel can be dried down using **SOP 22161** ***Invitrogen Gel Drying***.

6.0 References and Related Documents

SOP 22101 *SDS-PAGE Gel Electrophoresis Using Tris-Glycine Gel*

SOP 22161 *Invitrogen Gel Drying*

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

SOP 22702 *Solutions Used in BQC*

Electrophoresis Chapter 10 in Current Protocols in Protein Science, 1995

7.0 Change Summary