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1.0 Purpose

This procedure describes a method for performing polyacrylamide gel electrophoresis using Tris-Glycine gels in the XCELL II Mini-Cell/SureLock apparatus.

2.0 Scope

Process Analytics/Quality Control (PA/QC) 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 of PA/QC has the authority to define this procedure.

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

3.3 PA/QC personnel are responsible for the performance of this procedure.

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

3.5 BQA is responsible for quality oversight of this operation.

4.0 Reagents

NOTE: Record all solution preparations in the PA/QC Solutions Logbook as per ***SOP 22702 - Solutions Used in Process Analytics*.**

4.1 Novex® Tris-Glycine SDS Running Buffer (10x) (Catalog Number LC2675, BDP PN 30018 or equivalent.) 1800 mL H2O plus 200 mL (10x) Tris-Glycine SDS Running Buffer.

4.2 Novex® Tris-Glycine SDS Sample Buffer (2x) (Catalog Number LC2676, BDP PN 30017 or **equivalent**) with 2.5% Beta mercaptoethanol for a reducing buffer or without 2.5% Beta mercaptoethanol for a non-reducing buffer.

4.3 Molecular Weight Standards (Novex® or equivalent): Molecular weight standards are run with each gel as an internal calibration standard.

4.3.1 Novex® Mark 12 MW Standard (1x) (Catalog Number LC5677, BDP PN 30028) Range 200-2.5 kDa.

4.3.2 Novex® See Blue Plus 2 Standard (1x) (Catalog Number LC5925, BDP PN 30027).

4.4 Type 1 Laboratory Water, HPLC-grade or equivalent.

5.0 Equipment

- Novex® XCell SureLock® Mini-Cell (EI0001) Apparatus (Includes lower buffer chamber, buffer core with platinum electrodes, gold terminals and silicone rubber gaskets, buffer dam, cell lid with cables, wedge).
- Novex® PowerEase 500 Power Supply (EI8600).
- Calibrated Pipetors (10 µL, 20 µL, and 100 µL), Pipet tips, 1 µL to 250 µL capacity, BDP PN 21767, and Novex® Gel-loading tips (LC1001, BDP PN 20336 or equivalent.
- Novex® Pre-cast Gels - Pre-cast Gels of various acrylamide concentrations are available for optimal separation of proteins of different molecular weight ranges.
- 4-20% 1.0 mm gradient Tris-Glycine Gels (10-Pack: Catalog Number XP04200BOX. 2-Pack: XP04200PK2, BDP Part Number 30014 or equivalent).
- 4-12% 1.0 mm Gradient Tris-Glycine Gels (Catalog Number EC6035BOX or equivalent).
- 16% Tris-Glycine Gels (Catalog Number EC6495BOX, BDP Part Number 30482 or equivalent).
- Jule Inc., Pre-cast Gel, 7-20%, 0.75 mm gradient Tris-Glycine Gels (Catalog number 720D 0.75 NMCIOP), BDP PN 30774 or BDP approved equivalent.

  NOTE: If using 7-20% Tris-Glycine gels from Jule Inc., correct the lane assignment sheet to read Jule Inc., gels – 10 wells. Draw one line through Novex®, initial and date the change.

- 1.5 mL microcentrifuge tubes, BDP PN 20394.
- Gel staining container.
- Gel knife or spatula.

6.0 Standard and Sample Preparation

6.1 Pre-stained Molecular Weight Standards

No preparation is necessary for these standards. They can be loaded directly onto the gel. Follow package insert directions for volumes or prepare as follows.

6.1.1 Mark 12 MW standard. For Coomassie, load 10-15 µL/well of standard. For Silver-stain, dilute standard 1:20 in sample buffer, load 10-20 µL/well for mini-gels or load 1-2 µL of undiluted standard.

6.1.2 See Blue Plus 2 standard. For Western Blot, load 5-15 µL/well of standard. For Coomassie, load 10-15 µL/well of standard.

6.2 Sample Preparation

NOTE: Gloves must be worn both to protect the operator from the chemicals used and to protect the gel from protein on fingers which will show up on the gel using silver stain.

6.2.1 Dilute samples 1:1 with the Tris-Glycine 2X sample buffer. A typical sample preparation would be as follows: 20 µL of sample and 20 µL of 2x-sample buffer.

NOTE: Depending on a sample's original concentration, a dilution may need to be made using PBS, prior to diluting the sample (1:1) with the Tris-Glycine 2x sample buffer. This will bring the sample's concentration to an adequate amount to ensure that the sample protein concentration will not be overloaded.

6.2.2 Vortex the samples briefly (5-10 seconds). If a sample needs to be boiled, place the sample in a boiling water bath to denature the compound. The heat is turned off and the samples are incubated for 4 minutes. A heating block can also be used for sample denaturing. If a temperature other than 100°C is required, line out "100°C x 4 min" and record the specific temperature and time used on Form 22101-01 or 22101-02. Vortex samples briefly after heating.

6.2.3 For cleaning or rinse samples, load into the assigned wells 20 µL. For other samples with a known concentration, load approximately 0.1 µg-1.0 µg/well for silver stain and approximately 0.5 -10 µg/well for Coomassie stain.

7.0 Procedure

Requirements: Gloves must be worn.

7.1 Assembly of XCELL Mini-Cell/SureLock Apparatus

7.1.1 Cut open the gel cassette bag and drain away the buffer contents.

7.1.2 Peel off the tape covering the slot near the bottom of the gel cassette and wipe off excess buffer. The tape can be used to identify the samples by writing the QC Test Request number on the tape and placing it on the gel-staining container.

7.1.3 In one quick continuous motion, pull the comb out of the gel cassette exposing the wells.

7.1.4 Orient the buffer core in the lower buffer chamber so that the (+) and (-) symbols on the crosspiece face the front.

7.1.5 Insert the gel cassette(s) so that the notch that held the comb faces the buffer core. Two gels can be run simultaneously with one gel on either side of the buffer core. If only one gel is run, the square plastic buffer dam is used in place of the second gel cassette.

7.1.6 Insert the wedge into the lower buffer chamber and clamp into position to achieve a seal.

7.1.7 Pour 600 mL of running buffer into the lower buffer chamber and 200 mL of running buffer into the upper chamber. The upper buffer chamber is the void formed between two gel cassettes (or one gel cassette and the square plastic buffer dam). The running buffer must cover the sample wells.

7.1.8 Use a pipet to wash the sample wells with 1x running buffer. Leave the wells full of running buffer.

7.2 Sample and Standard Loading

7.2.1 Fill out the appropriate Novex® Gel Lane Assignment Form (22101-01 or 22101-02). On the Lane Assignment Form include the following:QC Test Request number; Project and Lot number; pipet number; Date and Person who ran the gel; Lot and Expiration date of gel; Lane assignment including sample name; Staining procedures; Sample buffer (reducing or non-reducing) lot number and expiration date; Standard name, lot number and expiration date; and Sample preparations and dilutions, if applicable.

7.2.2 Using the Novex® gel-loading pipet tips and following the lane assignment form, load the samples and standards by placing the pipet tip as close to the gel surface as possible without puncturing the gel, and slowly eject the sample or standard into its corresponding well.

7.3 Running Procedure

7.3.1 With the power OFF, place the lid on the buffer core ensuring that the electrodes are firmly seated with red to positive electrode (+) and black to negative electrode (-).

7.3.2 With the power OFF, connect the electrode wires to the power supply again with red to (+) and black to (-) electrodes.

7.3.3 Turn the power ON.

7.3.4 Press select for choices of gel type; select the gel type by using the arrow buttons.

7.3.5 Press select for number of gels to be run; use the arrow up key to select the number of gels.

7.3.6 Press select for gel running conditions. Select the running conditions and press start to begin.

NOTE: The typical running conditions for a Tris-Glycine SDS-PAGE is 1½ hours run time, 125v, 35mA, 5.0W. Running conditions may be adjusted for the needs of specific projects as long as running conditions are documented on the appropriate Lane Assignment form.

7.3.7 Record Date, Time, QC test request number, and running conditions in the appropriate Power Supply Equipment Logbook. (Refer to ***SOP 21531 – Equipment Logs***).

7.4 Disassembly

7.4.1 Once the run is completed, continue pressing stop to return to the first screen, and then turn the power off, and disconnect the electrodes. Be sure the power is off before disconnecting the electrodes.

7.4.2 Remove the lid and release the clamp on the wedge.

7.4.3 Remove the gel cassettes from the assembly and lay flat on a bench liner or paper towel.

7.4.4 Carefully insert the beveled edge of the knife or spatula in the gap between the two cassette plates and push down gently on the knife handle to separate the plates. Repeat on each side of the cassette until the plates are completely separated. Carefully remove and discard the top plate allowing the gel to remain on the bottom plate.

7.4.5 Use the sharp edge of the knife to cut off the bottom lip of the gel. Push down on the knife, and then repeat the motion across the gel.

7.4.6 Hold the cassette plate and gel over a container with the gel facing downward. Use the knife to carefully loosen one lower corner of the gel and allow the gel to peel away from the plate.

7.4.7 If the proteins are to be transferred to membranes, follow ***SOP 22179 - Western Transfer Blot***. If not, the gel should be fixed as soon as possible to prevent further migration of the proteins on the gel. Follow the desired staining or blotting procedure. For Coomassie Blue Stain, see ***SOP 22175 - Staining of Gels with Coomassie Blue R-250***; for Silver Stain see ***SOP 22177 - SilverXpress Silver Staining***. Perform densitometric evaluation of the gel according to ***SOP 22906 - Operation of the Gel-Pro 6.0 Analyzer Software for Densitometry***, if requested.

8.0 Documentation

8.1 Record the results on a PA QC Test Request, Form 22002-01. Once the gel has been fixed and stained (***SOP 22177 - SilverXpress Silver Staining*** or ***SOP 22175 - Staining of Gels with Coomassie Blue R-250***) and dried down (***SOP 22161 - Invitrogen Gel Drying***), attach it to the form by the Molecular Weight Marker indicators.

8.2 Sign and date the QC Test Request Form and attach the Lane Assignment Form.

8.3 Give the results to the PA/QC Supervisor for review and signature. The results then go to BQA for review and signature, and the original document is finally stored in the BQA Document Control Room.

9.0 References and Related Documents

9.1 Novex® XCELL II Mini-Cell Instructions Manual

9.2 Novex® Precast Gel Instructions

9.3 **SOP 22177** *SilverXpress Silver Staining*

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

9.5 **SOP 22161** *Invitrogen Gel Drying*

9.6	**SOP 22179**	*Western Transfer Blot*
9.7	**SOP 2290**6	*Operation of the Gel-Pro 6.0 Analyzer Software for Densitometry*
9.8	**SOP 22702**	*Solutions Used in Process Analytics*
9.9	**SOP 21531**	*Equipment Logs*
9.10	**Form 22101-01**	*BQC Novex® Gels-10 Wells, 4-20% - Tris Glycine/SDS Mark 12 MW Standard*
9.11	**Form 22101-02**	*BQC Novex® Gels – 10 Wells, 4-20% - Tris Glycine/SDS, See Blue Plus 2 Standard*
9.12	**Form 22101-03**	*MEF Equipment Logbook*