Title: SilverXpress Silver Staining

SOP Number: 22177

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Approval/Date:

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1.0 Purpose

This procedure describes a method for silver staining a polyacrylamide gel after electrophoresis.

2.0 Scope

Biopharmaceutical Quality Control (PA/QC) personnel will perform this procedure. Other Biopharmaceutical Development Program (BOP) personnel may use this protocol for development or in-process analysis.

3.0 Authority and Responsibility

3.1 The Director, Biopharmaceutical Quality Control (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 (BOA).

3.3 PA/QC personnel are responsible for the performance of this procedure. Laboratory personnel must wear gloves, safety glasses, and protective clothing while preparing and working with all the solutions in this procedure. Avoid breathing vapors and skin contact with Image Development Reagents. Read and understand all associated Material Safety Data Sheets (MSDS) for this procedure and dispose of all materials and samples per appropriate 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 (Invitrogen SilverXpress Silver Staining Kit)

4.1 Components of SilverXpress Kit (Catalog Number LC6100, BDP PN 30016, or BDP approved equivalent).

4.1.1 Sensitizing Solution.

4.1.2 Stainer A Solution.

4.1.3 Stainer B Solution.

4.1.4 Developing Solution.

4.1.5 Stopping Solution.

4.2 Reagent grade Methanol, BDP PN 30853, or BDP approved equivalent.

4.3 Reagent grade Acetic Acid, BDP PN 30860, or BDP approved equivalent.

4.4 Ultrapure Water or BDP Approved Equivalent.

5.0 Equipment

5.1 Plastic-coated stir bars.

5.2 Glass or plastic staining trays.

5.3 Shaker table.

5.4 Graduated cylinders.

5.5 Disposable pipettes.

5.6 Lab gloves.

6.0 Procedure

NOTE: Preparing each solution as per ***SOP 22702 – Solutions Used in Process Analytics***. All solutions should be properly disposed of in a chemical waste container. The final volume of each solution can be adjusted as needed as long as the component ratios remain unchanged.

(This procedure pertains to Tris-Glycine and NuPAGE Bis-Tris Gels. Refer to the Invitrogen protocol for other types of gels).

6.1 Fixing Step – (10 minutes)

Prepare the **Fixing Solution** as follows.

Reagent Grade Methanol	300 mL
Reagent Grade Acetic Acid	100 mL
High Purity Water	600 mL
Total	1 L

After gel electrophoresis, place gels in 200 mL of the Fixing Solution in a staining tray. With gentle agitation, fix the gels for 10 minutes (This is for both Tris-Glycine and NUPAGE Gels). Refer to the SilverXpress protocol table in the staining kit instructions for fixing times and solution volumes for different types of gels.

6.2 Sensitizing Step - Tris-Glycine (2 x 10 minutes) and NuPAGE Bis-Tris (2 x 30 minutes). Prepare Sensitizing Solution as follows.

High Purity Water	105 mL
Reagent Grade Methanol	100 mL
Sensitizer	5 mL
Total	200 mL

Decant the Fixing Solution from the staining tray. Add 100 mL of the sensitizing solution for 10 or 30 minutes (depending on gel type) with gentle agitation. After 10 or 30 minutes, decant the solution and replace with 100 mL of fresh sensitizing solution for an additional 10 or 30 minutes. It is important to note that the gels can stay in the sensitizing step overnight if necessary.

NOTE: The final volume of the solution containing Methanol and water reflect a volume shrinkage which occurs when these reagents are mixed. Do not adjust volumes of components or final volume.

6.3 Wash Step – Tris-Glycine (2 X 5 minutes) and NuPAGE Bis-Tris (2 X 10 minutes)

Decant the sensitizing solution from the staining tray. Rinse the gel with approximately 200 mL of high purity water for 5 or 10 minutes, with gentle agitation. After 5 or 10 minutes decant the water and replace it with 200 mL of fresh, high purity water for an additional 5 or 10 minutes.

6.4 Staining Step – (15 minutes)

Prepare **Staining Solution** as follows.

Stainer A	5 mL
Stainer B	5 mL
High Purity Water	90 mL
Total	100 mL

Decant the high purity water from the staining tray and add 100 mL staining solution for 15 minutes with gentle agitation.

6.5 Wash Step – (2 x 5 minutes)

Decant the staining solution and add 200 mL of high purify water to the staining tray and agitate gently. After 5 minutes, decant the water and replace it with 200 mL fresh, high purity water for an additional 5 minutes.

6.6 Developing Step – (3-15 minutes)

Prepare **Developing Solution** as follows.

Developer	5 mL
High Purity Water	95 mL
Total	100 mL

Decant the high purity water and add 100 mL of developing solution to the staining tray and agitate gently. The gel should develop in 3-15 minutes. The gel is fully developed when all the markers of the molecule weight standard are visible.

6.7 Stopping Step – (10 minutes)

After the gel has developed fully, add 5 mL stop solution directly to the developing solution in the staining tray. Agitate the gel gently for 10 minutes.

6.8 Wash – (3 x 10 minutes)

Decant the stop solution and add 200 mL high purity water for 10 minutes with gentle agitation. Decant the water and repeat 2 more times with fresh water. The gel is now finished staining and can be dried down (refer to ***SOP 22161 - Novex Gel Drying***). If densitometry is required, refer to ***SOP 22906 - Operation of the Gel-Pro Analyzer Software for Densitometry***.

7.0 Documentation

7.1 Record the results on the PA/QC Test Request, Form 22002-01.

7.2 Sign and date the PA/QC Request Form and attach the Lane Assignment Form.

7.3 Give results to the PA/QC Supervisor for review and signature.

8.0 References

8.1 SilverXpress Silver Stain Protocol.

8.2 SOP 22161 – Novex Gel Drying.

8.3 SOP 22702 - Solutions Used in Process Analytics.

8.4 SOP 22906 - Operation of the Gel-Pro Analyzer Software for Densitometry.

9.0 Attachments - SilverXpress Quick Reference

Attachment

SilverXpress Quick Reference (page 1 of 2)

QUICK REFERENCE

novex® by life technologies™

SilverXpress® Silver Staining Protocols

Revision Date 17 October 2012 **Catalog Number** LC6100 MAN0005695

WARNING! Before handling, read all applicable Safety Data Sheets (SDSs) at www.lifetechnologies.com/support.

The following protocol is for one mini-gel, 1.0-mm thick.

- For 1.5-mm thick mini-gels, double all incubation times.
- For 1.0-mm thick Novex® midi-gels, use ~1.5 times the volume of reagents recommended for a mini-gel.
- For detailed instructions and troubleshooting, refer to the instruction manual included with the kit.
- For samples reduced with DTT, use the procedure for NuPAGE® Bis-Tris Gels.

Step	Reagents	• Tris-Glycine Gels • NuPAGE® Tris-Acetate Gels	• Tricine Gels • NuPAGE® Bis-Tris Gels (or Samples reduced with DTT)
FIX	Ultra Pure Water 90 mL Methanol 100 mL Acetic Acid 20 mL Final Volume:* 200 mL	1 200 mL 10 min ☐	1 200 mL 10 min ☐
SENSITIZE	Ultra Pure Water 105 mL Methanol 100 mL Sensitizer 5 mL Final Volume:* 200 mL	2 100 mL 10 min ☐ 3 100 mL 10 min ☐	2 100 mL 30 min ☐ 3 100 mL 30 min ☐
WASH	Ultra Pure Water 400 mL	4 200 mL 5 min ☐ 5 200 mL 5 min ☐	NuPAGE® Tricine Bis-Tris 4 200 mL 5 min 10 min ☐ 5 200 mL 5 min 10 min ☐
STAIN	Stainer A 5 mL Stainer B 5 mL Ultra Pure Water 90 mL Final Volume: 100 mL	6 100 mL 15 min ☐	6 100 mL 15 min ☐
WASH	Ultra Pure Water 400 mL	7 200 mL 5 min ☐ 8 200 mL 5 min ☐	7 200 mL 5 min ☐ 8 200 mL 5 min ☐
DEVELOP	Developer 5 mL Ultra Pure Water 95 mL Final Volume: 100 ml	9 100 mL 3–15 min ☐	9 100 ml 3–15 min ☐
STOP	Stopper 5 mL Add directly to the developing solution.	10 5 mL 10 min ☐	10 5 mL 10 min ☐
WASH	Ultra Pure Water 600 mL	11 200 mL 10 min ☐ 12 200 mL 10 min ☐ 13 200 mL 10 min ☐	11 200 mL 10 min ☐ 12 200 mL 10 min ☐ 13 200 mL 10 min ☐

*The final volumes of solutions containing methanol and water account for a volume shrinkage that occurs when these reagents are mixed. Do not adjust volumes of components or final volume.

For research use only. Not for use in diagnostic procedures.

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Attachment (continued)

SilverXpress Quick Reference (page 2 of 2)

The following protocol is for one mini-gel, 1.0-mm thick.

- For 1.5-mm thick mini-gels, double all incubation times.
- For detailed instructions and troubleshooting, refer to the instruction manual included in the kit.
- For samples reduced with DTT, use the procedure for NuPAGE® Bis-Tris Gels (other side).

Step	Reagents	• IEF Gels • Native Gels	• TBE Gels • TBE-Urea Gels
FIX	Ultra Pure Water 200 mL TCA 24 g Sulphosalicylic Acid 7 g Final Volume: 200 mL	1 100 mL 10 min ☐ 2 100 mL 10 min ☐	1 200 mL 10 min ☐
SENSITIZE	Ultra Pure Water 105 mL Methanol 100 mL Sensitizer 5 mL Final Volume:* 200 mL	3 100 mL 30 min ☐ 4 100 mL 30 min ☐	2 100 mL 10 min ☐ 3 100 mL 10 min ☐
WASH	Ultra Pure Water 400 mL	5 200 mL 5 min ☐ 6 200 mL 5 min ☐	4 200 mL 5 min ☐ 5 200 mL 5 min ☐
STAIN	Stainer A 5 mL Stainer B 5 mL Ultra Pure Water 90 mL Final Volume: 100 mL	7 100 mL 15 min ☐	6 100 mL 30 min ☐
WASH	Ultra Pure Water 400 mL	8 200 mL 5 min ☐ 9 200 mL 5 min ☐	7 200 mL 5 min ☐ 8 200 mL 5 min ☐
DEVELOP	Developer 5 mL Ultra Pure Water 95 mL Final Volume: 100 mL	10 100 mL 3–15 min ☐	9 100 mL 3–15 min ☐
STOP	Stopper 5 mL Add directly to developing solution.	11 5 mL 10 min ☐	10 5 mL 10 min ☐
WASH	Ultra Pure Water 600 mL	12 200 mL 10 min ☐ 13 200 mL 10 min ☐ 14 200 mL 10 min ☐	11 200 mL 10 min ☐ 12 200 mL 10 min ☐ 13 200 mL 10 min ☐

*The final volumes of solutions containing methanol and water account for a volume shrinkage that occurs when these reagents are mixed. Do not adjust volumes of the components or the final volume.

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