

SOP Title: SilverXpress Silver Staining

SOP Number: 22177

Revision: 03

TABLE OF CONTENTS

1. PURPOSE	1
2. SCOPE	1
3. RESPONSIBILITIES	1
4. SAFETY.....	2
5. MATERIALS AND REAGENTS.....	2
6. EQUIPMENT	2
7. PROCEDURE.....	3
8. DOCUMENTATION AND RECORDS.....	5
9. REFERENCES AND RELATED DOCUMENTS.....	5
10. ATTACHMENTS	5

1. PURPOSE

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

2. 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. RESPONSIBILITIES

3.1 Director/ Biopharmaceutical Quality Control (PA/QC)

- Defines this procedure.

3.2 PC/QC personnel

- Provides training.
- Performs the procedure.
- Records and reviews data.

3.3 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight.

BIOPHARMACEUTICAL DEVELOPMENT PROGRAM

SOP Title: SilverXpress Silver Staining

SOP Number: 22177

Revision: 03

4. SAFETY

- 4.1 Laboratory personnel must wear gloves, safety glasses, and protective clothing while preparing and working with all the solutions in this procedure.
- 4.2 Avoid breathing vapors and skin contact with Image Development Reagents.
- 4.3 Read and understand all associated Material Safety Data Sheets (MSDS) for this procedure and dispose of all materials and samples per appropriate procedure.
- 4.4 All solutions should be properly disposed of in a chemical waste container.

5. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
30016	Components of SilverXpress Kit Catalog Number LC6100: Sensitizing Solution, Stainer A Solution, Stainer B Solution, Developing Solution, Stopping Solution.	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
30853	Reagent grade Methanol	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
30860	Reagent grade Acetic Acid	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
N/A	Ultrapure Water	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
N/A	Plastic-coated stir bars	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
N/A	Graduated cylinders	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
20100	Disposable pipettes	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
N/A	Glass or plastic staining trays	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
20764, 20765 20766	Lab gloves	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO

6. EQUIPMENT

- Shaker table
- Calibrated timer

SOP Title: SilverXpress Silver Staining

SOP Number: 22177

Revision: 03

7. PROCEDURE

NOTE: Preparing each solution as per SOP 22702 – Solutions Used in Process Analytics. 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).

7.1 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.

7.2 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.

SOP Title: SilverXpress Silver Staining

SOP Number: 22177

Revision: 03

7.3 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.

7.4 Staining Step – (15 minutes)

Prepare **Staining Solution** as follows.

Stainer A	5 mL
Stainer B	5 mL
<u>High Purity Water</u>	<u>90 mL</u>
Total	100 mL

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

7.5 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.

7.6 Developing Step – (3-15 minutes)

Prepare **Developing Solution** as follows.

Developer	5 mL
<u>High Purity Water</u>	<u>95 mL</u>
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.

7.7 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.

SOP Title: SilverXpress Silver Staining

SOP Number: 22177

Revision: 03

7.8 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**.

8. DOCUMENTATION AND RECORDS

Record the results on the PA/QC Test Request via the electronic QCTR System, using **SOP 22981 Submission, Testing, Review, and Approval of Quality Control Test Request Submissions using the BDP QCTR Submission Application**.

9. REFERENCES AND RELATED DOCUMENTS

Document Number	Title
22002-01	PA/QC Test Request
22161	Novex Gel Drying
22702	Solutions Used in Process Analytics
22906	Operation of the Gel-Pro Analyzer Software for Densitometry
22981	Submission, Testing, Review, and Approval of Quality Control Test Request Submissions using the BDP QCTR Submission Application

10. ATTACHMENTS

Attachment 1 SilverXpress Quick Reference

SOP Title: SilverXpress Silver Staining

SOP Number: 22177

Revision: 03

Attachment 1 SilverXpress Quick Reference (page 1 of 2)

QUICK REFERENCE



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 <input type="checkbox"/>	1 200 mL 10 min <input type="checkbox"/>
SENSITIZE	Ultra Pure Water 105 mL Methanol 100 mL Sensitizer 5 mL Final Volume:* 200 mL	2 100 mL 10 min <input type="checkbox"/> 3 100 mL 10 min <input type="checkbox"/>	2 100 mL 30 min <input type="checkbox"/> 3 100 mL 30 min <input type="checkbox"/>
WASH	Ultra Pure Water 400 mL	4 200 mL 5 min <input type="checkbox"/> 5 200 mL 5 min <input type="checkbox"/>	NuPAGE® Tricine Bis-Tris 4 200 mL 5 min 10 min <input type="checkbox"/> 5 200 mL 5 min 10 min <input type="checkbox"/>
STAIN	Stainer A 5 mL Stainer B 5 mL Ultra Pure Water 90 mL Final Volume: 100 mL	6 100 mL 15 min <input type="checkbox"/>	6 100 mL 15 min <input type="checkbox"/>
WASH	Ultra Pure Water 400 mL	7 200 mL 5 min <input type="checkbox"/> 8 200 mL 5 min <input type="checkbox"/>	7 200 mL 5 min <input type="checkbox"/> 8 200 mL 5 min <input type="checkbox"/>
DEVELOP	Developer 5 mL Ultra Pure Water 95 mL Final Volume: 100 mL	9 100 mL 3-15 min <input type="checkbox"/>	9 100 mL 3-15 min <input type="checkbox"/>
STOP	Stopper 5 mL Add directly to the developing solution.	10 5 mL 10 min <input type="checkbox"/>	10 5 mL 10 min <input type="checkbox"/>
WASH	Ultra Pure Water 600 mL	11 200 mL 10 min <input type="checkbox"/> 12 200 mL 10 min <input type="checkbox"/> 13 200 mL 10 min <input type="checkbox"/>	11 200 mL 10 min <input type="checkbox"/> 12 200 mL 10 min <input type="checkbox"/> 13 200 mL 10 min <input type="checkbox"/>

*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.



SOP Title: SilverXpress Silver Staining
SOP Number: 22177
Revision: 03

Attachment 1 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 <input type="checkbox"/> 2 100 mL 10 min <input type="checkbox"/>	1 200 mL 10 min <input type="checkbox"/>
SENSITIZE	Ultra Pure Water 105 mL Methanol 100 mL Sensitizer 5 mL Final Volume:* 200 mL	3 100 mL 30 min <input type="checkbox"/> 4 100 mL 30 min <input type="checkbox"/>	2 100 mL 10 min <input type="checkbox"/> 3 100 mL 10 min <input type="checkbox"/>
WASH	Ultra Pure Water 400 mL	5 200 mL 5 min <input type="checkbox"/> 6 200 mL 5 min <input type="checkbox"/>	4 200 mL 5 min <input type="checkbox"/> 5 200 mL 5 min <input type="checkbox"/>
STAIN	Stainer A 5 mL Stainer B 5 mL Ultra Pure Water 90 mL Final Volume: 100 mL	7 100 mL 15 min <input type="checkbox"/>	6 100 mL 30 min <input type="checkbox"/>
WASH	Ultra Pure Water 400 mL	8 200 mL 5 min <input type="checkbox"/> 9 200 mL 5 min <input type="checkbox"/>	7 200 mL 5 min <input type="checkbox"/> 8 200 mL 5 min <input type="checkbox"/>
DEVELOP	Developer 5 mL Ultra Pure Water 95 mL Final Volume: 100 mL	10 100 mL 3-15 min <input type="checkbox"/>	9 100 mL 3-15 min <input type="checkbox"/>
STOP	Stopper 5 mL Add directly to developing solution.	11 5 mL 10 min <input type="checkbox"/>	10 5 mL 10 min <input type="checkbox"/>
WASH	Ultra Pure Water 600 mL	12 200 mL 10 min <input type="checkbox"/> 13 200 mL 10 min <input type="checkbox"/> 14 200 mL 10 min <input type="checkbox"/>	11 200 mL 10 min <input type="checkbox"/> 12 200 mL 10 min <input type="checkbox"/> 13 200 mL 10 min <input type="checkbox"/>

*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.

Limited Product Warranty
 Life Technologies Corporation and/or its affiliates warrant their products as set forth in the Life Technologies (General) Terms and Conditions of Sale found at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

© 2015 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

DISCLAIMER
 LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATES DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATES BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE, FOR ANY SPECIAL, INCIDENTAL, PUNITIVE, OR CONSEQUENTIAL DAMAGES, IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

For support visit lifetechnologies.com/support
lifetechnologies.com

