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SilverXpress™ silver staining kit

USER GUIDE

For sensitive silver staining of proteins and nucleic acids

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For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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The information in this guide is subject to change without notice.

Contents

SilverXpress™ silver staining kit

For sensitive silver staining of proteins and nucleic acids

Kit contents and storage

Contents

The solutions included in the SilverXpress™ Silver Staining Kit are listed in the following table. Sufficient reagents are supplied to stain 25 mini-gels.

Item	Amount	Safety Information
Sensitizer	5 × 25 mL	Contains Glutaraldehyde
Stainer A	125 mL	Contains Silver Nitrate
Stainer B	5 × 25 mL	Contains Ammonium Hydroxide & Sodium Hydroxide
Developer	125 mL	Contains Formaldehyde & Citric Acid
Stopper	125 mL	Contains Citric Acid

Shipping and storage

The SilverXpress™ Silver Staining Kit is shipped at room temperature. Upon receipt, store the kit at 2℃ to 8℃. The kit is stable for 6 months when stored at 2℃ to 8℃.

Introduction

Product overview

About the kit

The SilverXpress™ Silver Staining Kit is a highly sensitive silver staining kit providing nanogram-level sensitivity with minimal background. The staining procedure requires less total time than standard Coomassie™ staining and is complete in a little over 1 hour.

The basic mechanism of silver staining is the reduction of silver nitrate to metallic silver at a protein band. The bands are visualized where the silver grain deposits. There are 2 types of silver staining processes: chemical development and photo development. The chemical development stains utilize ammonium hydroxide to form silver diamine complexes; the bands are visualized by acidification. The SilverXpress™ Silver Staining Kit is based on the chemical reduction of silver ions to metallic silver on a protein band.

General guidelines

Materials supplied by the user

- Staining Tray, 1 tray per gel
- Reagent Grade Methanol
- Reagent Grade Acetic Acid
- Ultra pure Water (18 megohm/cm resistance recommended)
- Rotary Shaker
- Graduated Cylinders (glass recommended)
- 10-mL Pipettes (disposable recommended)
- Bottles for solution preparation
- Latex or vinyl gloves
- Tricholoracetic acid (TCA) for IEF, TBE, and TBE-Urea gels
- Sulfosalicylic Acid (IEF, TBE, and TBE-Urea gels)

Note: To ensure safe and reliable operation, always use the SilverXpress™ Silver Staining Kit according to the protocol. Wear protective gloves and safety glasses when working in a laboratory environment.

Staining containers

- Use clean round containers and designate these containers for silver staining purposes only.
- Make sure that the container diameter and depth is sufficient to permit gel coverage with 100 mL of solution per gel. A container measuring 14 cm in diameter and 5.5 cm in height is ideal.
- The StainEase™ Gel Staining Tray is a 14.5 cm diameter tray with a clear polycarbonate outer container and lid and a removable white polycarbonate strainer insert. It is ideal for preventing gel breakage during multiple solution changes and for easily visualizing band development. See "Accessory products" on page 21 for ordering information.

Shaker

Set at 1 revolution per second.

Water

- Always use ultra pure water (18 megohm/cm resistance recommended) for preparing solutions, gel rinses, and container washing.
- Water of less than 18 megohm/cm may increase the background or impair band development.
- Store ultra pure water in glass containers. Water stored in plastic containers can become contaminated.

Solutions

- Avoid cross contamination of kit reagents especially between Stainer A and Stainer B.
- Use Teflon™-coated stir bars and clean glass containers to prepare reagents.
- Make all solutions fresh.
- Prepare the Fixing, Sensitizing and Staining Solutions under a hood. Cap solutions after use to keep exposure to external environment to a minimum.

Detection

- Detection is in the nanogram levels. You can detect 0.86 ng of BSA on a 1.0-mm Novex™ 4–20% Tris-Glycine gel.
- Samples reduced with dithiothreitol (DTT) will be less sensitive than non-reduced samples. To obtain optimal sensitivity with DTT reduced samples, use the Tricine protocol in "Staining tricine gels, NuPAGE™ novex Bis-Tris Gels, or reduced samples" on page 11.
- Non-reduced samples will have a slightly higher background than reduced samples. This will not affect detection limits.
- Silver staining sensitivity often depends on the specific protein structure. If dealing with a protein which is difficult to silver stain, Coomassie™ staining prior to silver staining may reduce the protein-to-protein variability and may result in higher sensitivity.
- If you are using SilverXpress™ to stain a gel which has been Coomassie™ Stained, omit the Fixing step in the SilverXpress™ protocol and proceed directly to the Sensitizing step.

Gel handling

- Be sure to wear rubber gloves that have been rinsed with deionized water while handling gels.
- Do not touch the gel with bare hands or metal objects and do not put pressure on gels while handling or changing solutions.

Protocols

Choose the procedure appropriate to your gel and sample type (see “Methods“ on page 9).

- Prepare all solutions according to the relevant table before proceeding with the protocol.
- To achieve best results, be sure to keep the volume of all solutions and the incubation time of all steps exactly as given in the protocol. Changes in the protocol can result in high background or poor band development.
- Use a grease pencil to mark off completed steps on your Quick Reference Guide included in the kit.

Protein standards

We recommend using Mark12™ Unstained Standard (see “Accessory products“ on page 21 for ordering information) for NuPAGE™, Tris-Glycine, and Tricine gels. Dilute Mark12™ Unstained Standard to 1:20 and load 5 µL per lane.

Staining NuPAGE™ novex Tris-Acetate and Tris-Glycine gels

Introduction

This method is recommended for staining all NuPAGE™ Novex™ Tris-Acetate and Tris-Glycine gels. For samples reduced with DTT, see "Staining tricine gels, NuPAGE™ novex Bis-Tris Gels, or reduced samples" on page 11.

Prepare solutions

Prepare the solutions as described in the following table.

Note: The final volumes of solutions containing both methanol and water reflect a volume shrinkage which occurs when these 2 reagents are mixed. Do not adjust volumes of components or final volume.

Solutions	Component	1 Gel	2 Gels	3 Gels	4 Gels
Fixing Solution	Ultra pure water	90 mL	180 mL	270 mL	360 mL
	Methanol	100 mL	200 mL	300 mL	400 mL
	Acetic Acid	20 mL	40 mL	60 mL	80 mL
Sensitizing Solution	Ultra pure water	105 mL	210 mL	315 mL	420 mL
	Methanol	100 mL	200 mL	300 mL	400 mL
	Sensitizer	5 mL	10 mL	15 mL	20 mL
Staining Solution	Stainer A	5 mL	10 mL	15 mL	20 mL
	Stainer B	5 mL	10 mL	15 mL	20 mL
	Ultra pure water	90 mL	180 mL	270 mL	360 mL
Developing Solution	Ultra pure water	95 mL	190 mL	285 mL	380 mL
	Developer	5 mL	10 mL	15 mL	20 mL
Stopping Solution	Stopper	5 mL	10 mL	15 mL	20 mL

Procedure

Follow the following procedure for 1-mm or 1.5-mm mini-gel as described in the following table.

Note: Gels may be stored in the second Sensitizing Solution overnight, if desired.

Step	Solution	Vol/Gel	Time	
			1-mm Gel	1.5-mm Gel
1	Fix the gel in Fixing Solution.	200 mL	10 min	20 min
2A	Decant the Fixing Solution and incubate the gel in 2 changes of Sensitizing Solution.	100 mL	10 min	20 min
2B		100 mL	10 min	20 min
3A	Decant the Sensitizing Solution and rinse the gel twice with ultra pure water.	200 mL	5 min	10 min
3B		200 mL	5 min	10 min
4	Incubate the gel in Staining Solution.	100 mL	15 min	30 min
5A	Decant the Staining Solution and rinse the gel twice with ultra pure water.	200 mL	5 min	10 min
5B		200 mL	5 min	10 min
6	Incubate the gel in Developing Solution.	100 mL	3–15 min	3–15 min
7	Add the Stopping Solution directly to the gel when the desired staining intensity is reached.	5 mL	10 min	20 min
8A	Decant the Stopping Solution and wash the gel 3 times in ultra pure water.	200 mL	10 min	20 min
8B		200 mL	10 min	20 min
8C		200 mL	10 min	20 min

Staining tricine gels, NuPAGE™ novex Bis-Tris Gels, or reduced samples

Introduction

This method is recommended for staining all NuPAGE™ Novex™ Bis-Tris Gels, Tricine Gels, or samples reduced with DTT.

Preparing solutions

Prepare the solutions as described in the following table.

Note: The final volumes of solutions containing both methanol and water reflect a volume shrinkage which occurs when these 2 reagents are mixed. Do not adjust volumes of components or final volume.

Solutions	Component	1 Gel	2 Gels	3 Gels	4 Gels
Fixing Solution	Ultra pure water Methanol Acetic Acid	90 mL 100 mL 20 mL	180 mL 200 mL 40 mL	270 mL 300 mL 60 mL	360 mL 400 mL 80 mL
Sensitizing Solution	Ultra pure water Methanol Sensitizer	105 mL 100 mL 5 mL	210 mL 200 mL 10 mL	315 mL 300 mL 15 mL	420 mL 400 mL 20 mL
Staining Solution	Stainer A Stainer B Ultra pure water	5 mL 5 mL 90 mL	10 mL 10 mL 180 mL	15 mL 15 mL 270 mL	20 mL 20 mL 360 mL
Developing Solution	Ultra pure water Developer	95 mL 5 mL	190 mL 10 mL	285 mL 15 mL	380 mL 20 mL
Stopping Solution	Stopper	5 mL	10 mL	15 mL	20 mL

Procedure

Follow the following procedure for 1-mm or 1.5-mm mini-gels as described in the table.

Note: Gels may be stored in the second Sensitizing Solution overnight, if desired.

Step	Solution	Vol/Gel	Time	
			1-mm Gel	1.5-mm Gel
1	Fix the gel in Fixing Solution.	200 mL	10 min	20 min
2A	Decant the Fixing Solution and incubate the gel in two changes of Sensitizing Solution.	100 mL	30 min	60 min
2B		100 mL	30 min	60 min
3A	**For Tricine gels and reduced samples** Decant the Sensitizing Solution and rinse the gel twice with ultra pure water.	200 mL	5 min	10 min
3B		200 mL	5 min	10 min
3A	**For NuPAGE™ Bis-Tris Gels** Decant the Sensitizing Solution and rinse the gel twice with ultra pure water.	200 mL	10 min	20 min
3B		200 mL	10 min	20 min
4	Incubate the gel in Staining Solution.	100 mL	15 min	30 min
5A	Decant the Staining Solution and rinse the gel twice with ultra pure water.	200 mL	5 min	10 min
5B		200 mL	5 min	10 min
6	Incubate the gel in Developing Solution.	100 mL	3–15 min	3–15 min

Step	Solution	Vol/Gel	Time	
			1-mm Gel	1.5-mm Gel
7	Add the Stopping Solution directly to the gel when the desired staining intensity is reached.	5 mL	10 min	20 min
8A	Decant the Stopping Solution and wash the gel three times in ultra pure water.	200 mL	10 min	20 min
8B		200 mL	10 min	20 min
8C		200 mL	10 min	20 min

Staining TBE and TBE-Urea gels

Introduction

This method is recommended for staining all Novex™ TBE and TBE-Urea gels.

Preparing solutions

Prepare the solutions as described in the following table.

Note: The final volumes of solutions containing both methanol and water reflect a volume shrinkage which occurs when these 2 reagents are mixed. Do not adjust volumes of components or final volume.

Solutions	Component	1 Gel	2 Gels	3 Gels	4 Gels
Fixing Solution[1]	Ultra pure water TCA Sulphosalicylic Acid	200 mL 24 g 7 g	300 mL 36 g 10.5 g	400 mL 48 g 14 g	500 mL 60 g 17.5 g
Sensitizing Solution	Ultra pure water Methanol Sensitizer	105 mL 100 mL 5 mL	210 mL 200 mL 10 mL	315 mL 300 mL 15 mL	420 mL 400 mL 20 mL
Staining Solution	Stainer A Stainer B Ultra pure water	5 mL 5 mL 90 mL	10 mL 10 mL 180 mL	15 mL 15 mL 270 mL	20 mL 20 mL 360 mL
Developing Solution	Ultra pure water Developer	95 mL 5 mL	190 mL 10 mL	285 mL 15 mL	380 mL 20 mL
Stopping Solution	Stopper	5 mL	10 mL	15 mL	20 mL

[1] Fixing Solution should not be stored for over 1 month. If solution turns pink, remake it.

Note: To increase the sensitivity to 0.3 ng of double strand DNA for 20% TBE Gels, modify the protocol described as follows:

- Eliminate the fixing step
- Incubate the gel in the Sensitizing Solution containing 2 mL sensitizer and 189 mL ultra pure water (no methanol) **once** for 20 minutes

Procedure

Follow the procedure for 1-mm or 1.5-mm mini-gel as described in the table below.
Note: Gels may be stored in the second Sensitizing Solution overnight, if desired.

Step	Solution	Vol/Gel	Time	
			1-mm Gel	1.5-mm Gel
1	Fix the gel in Fixing Solution.	200 mL	10 min	20 min
2A	Decant the Fixing Solution and incubate the gel in two changes of Sensitizing Solution.	100 mL	10 min	20 min
2B		100 mL	10 min	20 min
3A	Decant the Sensitizing Solution and rinse the gel twice with ultra pure water.	200 mL	5 min	10 min
3B		200 mL	5 min	10 min
4	Incubate the gel in Staining Solution.	100 mL	30 min	60 min
5A	Decant the Staining Solution and rinse the gel twice with ultra pure water.	200 mL	5 min	10 min
5B		200 mL	5 min	10 min
6	Incubate the gel in Developing Solution.	100 mL	3–15 min	3–15 min
7	Add the Stopping Solution directly to the gel when the desired staining intensity is reached.	5 mL	10 min	20 min
8A	Decant the Stopping Solution and wash the gel three times in ultra pure water.	200 mL	10 min	20 min
8B		200 mL	10 min	20 min
8C		200 mL	10 min	20 min

Staining IEF gels

Introduction

This method is recommended for staining all Novex™ IEF gels.

Preparing solutions

Prepare the solutions as described in the following table.

Note: The final volumes of solutions containing both methanol and water reflect a volume shrinkage which occurs when these 2 reagents are mixed. Do not adjust volumes of components or final volume.

Solutions	Component	1 Gel	2 Gels	3 Gels	4 Gels
IEF Fixing Solution	Ultra pure water TCA Sulphosalicylic Acid	200 mL 24 g 7 g	300 mL 36 g 10.5 g	400 mL 48 g 14 g	500 mL 60 g 17.5 g
Sensitizing Solution	Ultra pure water Methanol Sensitizer	105 mL 100 mL 5 mL	210 mL 200 mL 10 mL	315 mL 300 mL 15 mL	420 mL 400 mL 20 mL
Staining Solution	Stainer A Stainer B Ultra pure water	5 mL 5 mL 90 mL	10 mL 10 mL 180 mL	15 mL 15 mL 270 mL	20 mL 20 mL 360 mL
Developing Solution	Ultra pure water Developer	95 mL 5 mL	190 mL 10 mL	285 mL 15 mL	380 mL 20 mL
Stopping Solution	Stopper	5 mL	10 mL	15 mL	20 mL

Procedure

Follow the procedure for 1-mm or 1.5-mm mini-gel as described in the table below.
Note: Gels may be stored in the second Sensitizing Solution overnight, if desired.

Step	Solution	Vol/Gel	Time	
			1 mm Gel	1.5 mm Gel
1A	Incubate the gel in two changes of IEF Fixing Solution.	100 mL	10 min	20 min
1B		100 mL	10 min	20 min
2A	Decant the Fixing Solution and incubate the gel in two changes of Sensitizing Solution.	100 mL	30 min	60 min
2B		100 mL	30 min	60 min
3A	Decant the Sensitizing Solution and rinse the gel twice with ultra pure water.	200 mL	5 min	10 min
3B		200 mL	5 min	10 min
4	Incubate the gel in Staining Solution.	100 mL	15 min	30 min
5A	Decant the Staining Solution and rinse the gel twice with ultra pure water.	200 mL	5 min	10 min
5B		200 mL	5 min	10 min
6	Incubate the gel in Developing Solution.	100 mL	3–15 min	3–15 min
7	Add the Stopping Solution directly to the gel when the desired staining intensity is reached.	5 mL	10 min	20 min
8A	Decant the Stopping Solution and wash the gel three times in ultra pure water.	200 mL	10 min	20 min
8B		200 mL	10 min	20 min
8C		200 mL	10 min	20 min

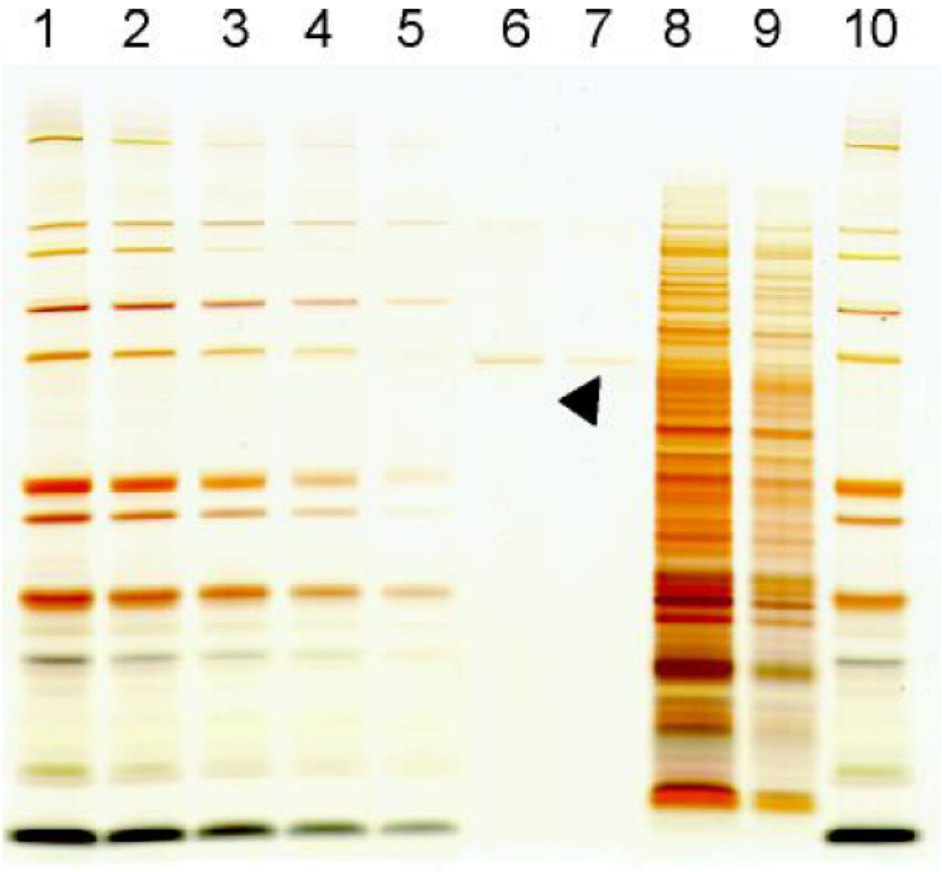
Results

Expected results

Using the silver staining protocol described in this manual, you should be able to detect:

- > 0.8 ng of protein and the gel should have a light background
- < 0.5 ng of 50 bp DNA

An example of a gel stained with the SilverXpress™ Silver Staining Kit is shown below.



Legend for the gel:

Different dilutions of proteins and standards were electrophoresed on a NuPAGE™ Novex™ 4–12% Bis-Tris Gel. The gel was stained with SilverXpress™ Silver Staining Kit as described in this manual.

Lane 1, 10:	Mark12™ Standard diluted 1:4
Lane 2:	Mark12™ Standard diluted 1:8
Lane 3:	Mark12™ Standard diluted 1:16
Lane 4:	Mark12™ Standard diluted 1:32
Lane 5:	Mark12™ Standard diluted 1:64

Lane 6:	1.6 ng BSA
Lane 7:	0.8 ng BSA (indicated by arrowhead)
Lane 8:	*E. coli* lysate diluted 1:20
Lane 9:	*E. coli* lysate diluted 1:80

Troubleshooting

Trouble-shooting

Review the information provided in the following table to troubleshoot your experiments.

Observation	Cause	Solution
Uneven gray or black background staining	Poor quality water used for preparing solutions or rinsing	Use ultra pure water of >18 megohm/cm resistance.
	Missed a rinse step	Do not skip or reduce rinse steps.
Gels have a yellow background	Contaminants from the sample wells entered the gel	Carefully rinse the sample wells with 5 or more changes of 1X running buffer prior to sample loading.
	Poor water quality	Use ultra pure water of >18 megohm/cm resistance.
	Contaminated equipment used to prepare reagents	Use glass columns and sterile pipettes to prepare reagents. Wash glassware thoroughly.
Poor or no image development	Low protein load	Increase the amount of protein load. Be sure to have at least of 1–5 ng protein on the gel.
	Poor water quality	Use ultra pure water of >18 megohm/cm resistance.
	Incorrect volumes of water used for rinses	Use exact volumes of all components and strictly adhere to the protocol.

Observation	Cause	Solution
Poor or no image development	Stainer or developer solution not prepared properly	• If you do not observe any bands within 5 minutes of adding the Developing Solution, add 5 mL of the Developer directly to the staining tray. • Make sure that the solutions are prepared correctly using ultra pure water.
Stained gels are too dark	Stopper not added at the appropriate time	Be sure to add the stopper slightly before the desired stain intensity is reached.
Negative staining	Protein band is overloaded	Decrease protein load per band. For an accurate control, dilute the Mark12™ Unstained Standard 1:20 with 1X sample buffer.

Frequently asked questions

If I need to stop in the middle of the silver staining procedure, at what point should this be done?

The sensitizing step is the optimal point to stop the procedure. You may leave the gel in the Sensitizing Solution during lunch or even overnight.

When using this Kit, it is difficult to tell if I am performing the steps correctly as the bands do not appear until the development stage. Do you have any hints?

Use the following visual cues as landmarks of a properly completed step.

1. The low percentage stacking gel appears whitish opaque as compared to the separating gel after the Sensitizing Step indicating that this step was performed correctly.
2. Mini-gels curl up into a cylinder and float on the surface during the first water wash after the Sensitizing Step.
3. When Stainer A and Stainer B are added together, a brown precipitate is formed, which is visible only momentarily. This brown “flash” is a good indicator that the staining solutions are mixed correctly. If the brown color does not revert to clear, discard the solutions, obtain clean glassware, and remix the solutions.

Appendix

Accessory products

Additional products

A large variety of products that can be used for staining and drying gels are available separately. Ordering information is provided in the following table. For more details on these products, visit **www.lifetechnologies.com** or contact Technical Support ().

Product	Quantity	Catalog no.
Mark12™ Unstained Standard	1 mL	LC5677
BenchMark™ Protein Ladder	2 × 250 µL	10747-012
StainEase™ Staining Tray	2/pack	NI2400
SilverQuest™ Silver Staining Kit	1 Kit	LC6070
SimplyBlue™ SafeStain™	1 L	LC6060
DryEase™ Mini gel Drying System	1 Kit	NI2387
Gel-Dry™ Drying Solution (1X)	500 mL	LC4025

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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