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life technologies

Instruction Manual

Colloidal Blue Staining Kit

For sensitive staining of protein gels

Catalog no. LC6025

Version F
011002
IM-6025

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General Information

Overview

The Colloidal Blue Staining Kit (formerly called Colloidal Coomassie® Stain) provides nanogram-level detection of proteins and water clear backgrounds without destaining. Using this stain you can detect <10 ng of BSA on a 4-20% 1.0 mm Tris-Glycine gel in an hour.

The Colloidal Blue Staining Kit is based on the work from Neuhoff et. al.(Electrophoresis 1988, 9, 255-262). This method is based on the colloidal properties of Coomassie® Blue dyes created in aqueous or methanolic solutions containing inorganic acids and high salt concentrations. The free dye in solution is greatly reduced due to the hydrophobic effect, resulting in low background staining and high affinity binding of the dye to the proteins fixed in the gel.

Features

The important features of the Colloidal Blue Staining Kit are listed below:

- Sensitive, consistent, and easy to use protein stain
- Five times more sensitive than traditional Coomassie® Blue staining techniques
- Requires only one change of solution
- Protein bands visible within an hour and water clear background is achieved after 7 hours

Shipping and Storage

The Colloidal Blue Staining Kit is shipped at room temperature. Upon receipt, store the kit at room temperature. The kit is stable for 1 year when stored at room temperature.

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General Information, Continued

Kit Contents

The solutions included in the Colloidal Blue Staining Kit are listed below. Sufficient reagents are supplied to stain 25 mini-gels. The Colloidal Blue Staining Kit contains Coomassie G-250.

Item	Amount	Safety Information
Stainer A	500 ml	Contains Ammonium Sulfate and Phosphoric Acid
Stainer B	125 ml	N/A

Product Qualification

Invitrogen qualifies the Colloidal Blue Staining Kit using the staining protocol described in this manual. Different dilutions of BSA are electrophoresed on a 1.0 mm, Novex® 4-20% Tris-Glycine gel. Functional criteria are:

- Colloidal Blue staining must detect at least 6 ng of BSA
- Background must be clear, uniform, and free from uneven staining or contaminant bands
- Sensitivity must be reached wtihin 7 ± 1 hour

General Guidelines

Introduction

Observe the following guidelines to obtain the best results with Colloidal Blue Staining Kit.

Materials Supplied by the User

You will need the following items on hand before using the Colloidal Blue Staining Kit:

- Round staining tray with capacity of at least 200 ml
- Reagent grade methanol
- Deionized water
- Rotary shaker
- Graduated cylinder
- Latex or vinyl gloves
- Tricholoroacetic acid (TCA ,required for isoelectric focusing procedure)
- Sulfosalicylic acid (required for isoelectric focusing procedure)
- Bleach (recommended for clean-up after staining)

Note

To ensure safe, and reliable operation, always use the Colloidal Blue Staining Kit according to the protocol. Wear protective gloves and safety glasses when working in a laboratory environment.

Staining Containers

- Use clean, round containers for staining
- Make sure container diameter is sufficient to permit gel coverage with 100 ml of solution
- To stain two gels in the same container, use a container with a diameter sufficient to permit coverage of two gels with 200 ml of solution

Shaker

Set shaker at 1 revolution per second.

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General Guidelines, Continued

Water

Deionized water is sufficient for solution preparation.

Detection

Detection is in nanogram amounts; less than 10 ng of BSA is routinely detected on a 4-20% 1.0 mm Novex®Tris-Glycine Gel.

Non-reduced samples stain slightly more intensely than reduced samples. Bands are visible after 1 hour in staining solution.

Solutions

- Prepare solutions fresh prior to staining
- You may mix the solutions directly in the staining dish
- Be sure to shake Stainer B prior to making solution

Background

- Background is higher in low percentage acrylamide gels due to penetration and trapping of colloids within the large pores of these gels
- Background may be removed by incubating the gel in 25% methanol solution until a clear background is obtained. Be aware that dye will also be partially removed from the bands.
- Prolonged incubation in >25% methanol results in complete destaining of protein bands and background.

Gel Drying

- Do not leave the colloidal stained gel in pre-treatment gel drying solutions, such as Gel-Dry™ Solution, for more than 5 minutes.
- Prolonged exposure to pre-treatment gel drying solutions will destain the gel completely.

Clean-Up

A 5% bleach solution will effectively remove the colloidal stain from plastic, porcelain and metal surfaces.

Staining NuPAGE® Novex Tris-Acetate, Tris-Glycine, Tricine, and Zymogram Gels

Introduction

This method is recommended for staining all NuPAGE® Novex Tris-Acetate, Tris-Glycine, Tricine, and Zymogram gels.

Preparing Staining Solution

Prepare the solutions as described in the table below. For best results, prepare the solution fresh prior to staining. Be sure to shake Stainer B solution before using.

Solution*	1 Gel	2 Gels	3 Gels	4 Gels
Deionized Water	55 ml	110 ml	165 ml	220 ml
Methanol	20 ml	40 ml	60 ml	80 ml
Stainer B	5 ml	10 ml	15 ml	20 ml
Stainer A	20 ml	40 ml	60 ml	80 ml

*When Stainer A and Stainer B are combined a precipitate may form which will dissolve within 30 seconds.

Procedure

1. Shake gel in staining solution for a minimum of 3 hours and a maximum of 12 hours.

   Note: Staining intensity does not vary significantly if left in stain for 3 hours or 12 hours.
2. Decant staining solution and replace with a minimum of 200 ml of deionized water per gel. Shake gel in water for at least 7 hours. Gel will have a clear background after 7 hours in water.
   Note: Gels can be left in water for up to 3 days without significant change in band intensity and background clarity.
3. For long-term storage (over 3 days), keep the gel in a 20% ammonium sulfate solution at 4°C.
4. For gel drying, see page 9.

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Staining Isoelectric Focusing (IEF) Gels

Introduction

This method is recommended for staining all Novex® IEF Gels.

Preparing Solutions

Prepare the Fixing Solution and the Staining Solutions as described in the table below. For best results, prepare the solution fresh prior to staining. Be sure to shake Stainer B solution before using.

Fixing Solution	1 Gel	2 Gels	3 Gels	4 Gels
Deionized Water	100 ml	200 ml	300 ml	400 ml
TCA	12 g	24 g	36 g	48 g
Sulfosalicylic acid	3.5 g	7 g	10.5 g	14 g

Staining Solution*	1 Gel	2 Gels	3 Gels	4 Gels
Deionized Water	58 ml	116 ml	174 ml	232 ml
Methanol	20 ml	40 ml	60 ml	80 ml
Stainer B	2 ml	4 ml	6 ml	8 ml
Stainer A	20 ml	40 ml	60 ml	80 ml

*When Stainer A and Stainer B are combined a precipitate may form which will dissolve within 30 seconds.

Procedure

1. Shake gel in Fixing Solution for 1 hour.
2. Decant Fixing Solution and replace with 100 ml/gel of IEF Staining Solution. Shake in Staining Solution for 30 minutes.
3. Decant staining solution and replace with a minimum of 200 ml of deionized water per gel. Shake gel in water for at least 7 hours. Gel will have a clear background after 7 hours in water. **Note**: Gels can be left in water for up to 3 days without significant change in band intensity and background clarity.
4. For long-term storage (over 3 days), keep the gel in a 20% ammonium sulfate solution at 4°C.
5. For gel drying, see page 9.

Staining NuPAGE® Novex Bis-Tris Gels

Introduction

This method is recommended for staining all NuPAGE® Novex Bis-Tris Gels and for staining some peptides (<20 kDa) on all types of gels.

Preparing Solutions

Prepare the Fixing Solution as described in the table below. For best results, prepare the solution fresh prior to staining. Be sure to shake Stainer B solution before using.

Prepare the Staining Solution as described below **without Stainer B.**

Fixing Solution	1 Gel	2 Gels	3 Gels	4 Gels
Deionized Water	40 ml	80 ml	120 ml	160 ml
Methanol	50 ml	100 ml	150 ml	200 ml
Acetic Acid	10 ml	20 ml	30 ml	40 ml

Staining Solution*	1 Gel	2 Gels	3 Gels	4 Gels
Deionized Water	55 ml	110 ml	165 ml	220 ml
Methanol	20 ml	40 ml	60 ml	80 ml
Stainer A	20 ml	40 ml	60 ml	80 ml
Stainer B	5 ml	10 ml	15 ml	20 ml

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Staining NuPAGE® Novex Bis-Tris Gels,
Continued

Procedure

1. Shake the gel in the Fixing Solution for 10 minutes at room temperature.
2. Shake the gel in the Staining Solution without Stainer B for 10 minutes at room temperature.
3. Add Stainer B to the existing Staining Solution in the proper volume as shown below.

Stainer B	1 Gel	2 Gels	3 Gels	4 Gels
	5 ml	10 ml	15 ml	20 ml

4. Shake gel in Staining Solution for a minimum of 3 hours and a maximum of 12 hours.

   Note: Protein bands begin to appear in 2-5 minutes. Staining intensity does not vary significantly if left in stain for 3 hours or 12 hours.
5. Decant Staining Solution and replace with 200 ml of deionized water per gel. Shake gel in water for at least 7 hours. Gel will have a clear background after 7 hours in water. **Note**: Gels can be left in water for up to 3 days without significant change in band intensity and background clarity.
6. For long-term storage (over 3 days), keep the gel in 20% ammonium sulfate solution at 4°C.

Drying Gels

For gel drying using the DryEase® Gel Drying System, use the protocol in the manual (IM-2080). You can download the manual from our Web site at www.invitrogen.com or contact Technical Service (see page 11). Do not leave the gel in Gel-Dry™ solution or any solution containing more than 20%alcohol for more than 5 minutes. This may result in loss of band intensity and detection limits may decrease.

Related Products

Additional Products

The table below lists additional products that may be used for staining or drying the gel.

Product	Quantity	Catalog no.
SilverQuest™ Silver Staining Kit	1 kit	LC6070
SimplyBlue™ SafeStain	1 L	LC6060
SilverXpress® Silver Staining Kit	1 kit	LC6100
DryEase® Mini-Gel Drying System	1 kit	NI2387
Gel-Dry™ Drying Solution (1X)	500 ml	LC4025
StainEase® Staining Tray	2/pack	NI2400
Mark 12™ Unstained Standard	1 ml	LC5677
BenchMark™ Protein Ladder	2 x 250 µl	10747-012
SeeBlue® Plus 2 Pre-Stained Standard	500 µl	LC5925
MultiMark® Multi-Colored Standard	500 µl	LC5725
BenchMark™ Pre-stained Protein Ladder	2 x 250 µl	10748-010
SERVA® Liquid Mix IEF Marker 3-10	500 µl	39212-01

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
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Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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Technical Service, Continued

MSDS Requests

1. To request an MSDS, go to www.invitrogen.com.
2. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
3. Follow instructions on the page and fill out all the required fields.
4. To request additional MSDSs, click the 'Add Another' button.
5. All requests will be faxed unless another method is selected.
6. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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