INSTRUCTIONS

GelCode® Blue Stain Reagent

PIERCE
a Perbio Science Company

3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

24590 24592

0714.2

Number	Description
24590	**GelCode® Blue Stain Reagent**, 500 ml, sufficient for 20 mini gels
24592	**GelCode® Blue Stain Reagent**, 3.5 liters, sufficient for 175 mini gels

Note: A convenient dispenser pump (Product No. 72300) for the 3.5 liter container is available free, upon request, with the purchase of Product No. 24592.

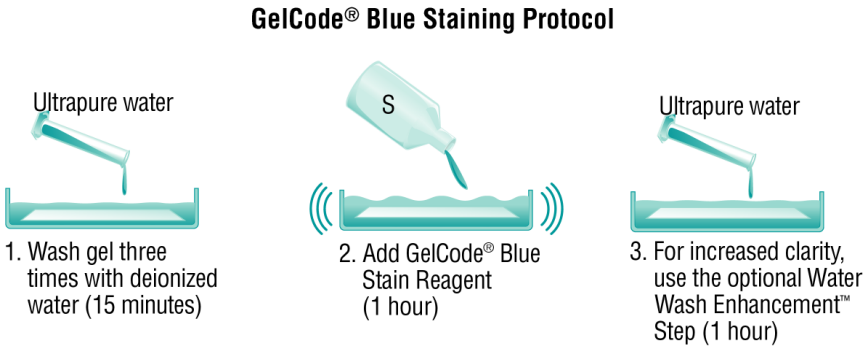
Storage: Upon receipt store product at 4°C. Product is stable up to 6 months at room temperature and longer than 1 year at 4°C. Product shipped at ambient temperature.

Introduction

GelCode® Blue Stain Reagent utilizes the colloidal properties of coomassie G-250 dye for protein staining on polyacrylamide gels. This unique reagent stains only protein and allows bands to be viewed directly on the gel during the staining process. After staining, a water equilibration step (Water Wash Enhancement™) further enhances staining sensitivity and yields a clear background.[1] With GelCode® Blue Stain there is no need for multi-step destaining procedures associated with other gel staining systems.[2]

Procedure Summary

Total assay time:
1 hour 15 minutes



Reagent Preparation

Mix the GelCode® Blue Stain Reagent solution immediately before use by gently inverting or tipping and swirling the bottle several times. Such mixing is especially important when using Product No. 24592 with a dispenser pump. Do not shake bottle to mix the solution.

Note: GelCode® Blue Stain Reagent contains additives that help to slow down the formation of dye-dye and dye-protein aggregates, which form in all coomassie dye-based protein staining reagents. If left undisturbed, the reagent will form visible dye-dye aggregates that settle in the bottom of the bottle. Fortunately, gentle mixing completely disperses these aggregates. Therefore, it is good practice to mix the stain reagent before pouring or dispensing to ensure that a homogeneous sample of the reagent is used.

Procedure for Staining Gels

A. Wash

- SDS-PAGE: After electrophoresis, place gel in a clean tray and rinse 3 x 5 minutes with 100-200 ml of ultrapure water. Alternatively, wash gel in 1-2 liters of ultrapure water with gentle shaking for 15 minutes.
- Native PAGE: Rinse gel with ultrapure water for 5 minutes.

Note: Gels electrophoresed with MOPS or MES running buffers must be prefixed with a 50% methanol and 7% acetic acid solution for 15 minutes and then washed with ultrapure water to remove fixing solution. GelCode® Blue Stain penetrates prefixed gels better than non-fixed gels and, therefore, standard SDS-PAGE gels may also be prefixed with good results.

B. Stain

Note: Mix the GelCode® Blue Stain Reagent solution immediately before use by gently inverting or tipping and swirling the bottle several times. Such mixing is especially important when using Product No. 24592 with a dispenser pump. Do not shake bottle to mix the solution.

Add 20 ml of GelCode® Blue Stain Reagent for an 8 x 10 cm mini gel. Additional reagent may be required if a large tray is being used. Gently shake tray and periodically monitor protein band development. Stain intensity reaches a maximum within approximately 1 hour. Gels may be stained overnight without increasing background.

Note: PhastGel® Gels may require increased staining times (2 hours to overnight) for optimal band development.

C. Destain (Water Wash Enhancement™ Step)

Replace Stain Reagent with ultrapure water. Several water changes for a 1-2 hour period may be necessary for optimal results. This step enhances stain sensitivity, as weak protein bands continue to develop.

Procedure for Staining Membranes

A. Wash

Place membrane containing transferred proteins in a clean tray and rinse for 1-2 minutes with ultrapure water.

B. Stain

Note: Mix the GelCode® Blue Stain Reagent solution immediately before use by gently inverting or tipping and swirling the bottle several times. Such mixing is especially important when using Product No. 24592 with a dispenser pump. Do not shake bottle to mix the solution.

Add 20 ml of GelCode® Blue Stain Reagent for an 8 x 10 cm membrane. Additional reagent may be required if a large tray is being used. Incubate on an orbital shaker for 2-5 minutes.

C. Destain

Destain with a solution of 50% methanol and 1% acetic acid for 4-10 minutes, replacing the solution 2-3 times.

Note: Before drying the membrane for preservation, rinse it with 10% methanol to prevent wrinkling.

Alternative Microwave Procedure For Gels

This microwave procedure results in faster staining with only a minimal loss in sensitivity (12 ng vs. 8 ng for the standard protocol). Bands will develop in approximately 30 minutes when using this method. This microwave procedure is optimized for standard 1 mm thick mini gels. Larger or thicker gels may require additional volumes of reagents and/or longer microwave and incubation times.

A. Wash

After electrophoresis, place gel into a microwavable tray containing 100 ml of ultrapure water, and microwave for 90 seconds. Discard water, replace with 100 ml of fresh ultrapure water, and microwave again for 90 seconds. Replace water with fresh ultrapure water and place on an orbital shaker for 5 minutes.

B. Stain

Note: Mix the GelCode® Blue Stain Reagent solution immediately before use by gently inverting or tipping and swirling the bottle several times. Such mixing is especially important when using Product No. 24592 with a dispenser pump. Do not shake bottle to mix the solution.

Discard water wash from gel and add 50 ml of GelCode® Blue Stain Reagent or sufficient volume to completely cover the gel, and microwave for 1 minute or until solution begins to boil. Do not let solution boil to evaporation. Place tray on an orbital shaker and incubate for 5 minutes.

C. Destain (Water Wash Enhancement™ Step)

Discard staining reagent and replace with 200 ml of ultrapure water. Incubate for 10 minutes on an orbital shaker.

Note: Frequently replacing water and washing for a longer time may increase band intensity (contrast with background).

Troubleshooting

Problem	Possible Cause	Solution
High Background	SDS interference	Wash gel extensively before the staining step
Reagent turns blue during staining process		
No band development	Gel is > 1 mm thick	Perform Water Wash Enhancement™ Step for 2-4 hours; alternatively, use thinner gels.

Related Pierce Products

24580	**MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes**
24602	**GelCode® SilverSNAP Silver Stain Kit**
24597	**GelCode® Color Silver Stain Kit**
26681	**BlueRanger™ Prestained Protein Molecular Weight Marker Mix**
28378	**BupH™ Tris-Glycine-SDS Buffer Packs**

Additional Information

A. Please visit the Pierce web site for additional information on this product including:

- Frequently Asked Questions
- Tech Tip protocol to Confirm Protein's Presence Before Staining with GelCode® Color Silver Stain

B. Performance Characteristics of GelCode® Blue Stain Reagent

Table 1. Relative absorption (OD/mm^2) of standard proteins separated by a 4%-20% gradient gel and stained with GelCode® Blue Stain Reagent.

Protein (ng)	Myosin H-chain (200 kD)	Phosphorylase b (97.4 kD)	Bovine serum albumin (68 kD)	Ovalbumin (43 kD)	Carbonic anhydrase (29 kD)	Lacto globulin (14.3 kD)	Lysozyme (18.4 kD)
3,000	3.5	5.1	9.4	17.6	10.3	19.5	14.8
2,000	2.7	3.8	7.3	14.2	8.4	16.0	11.8
1,000	1.6	2.0	3.6	8.0	4.9	9.4	7.0
500	1.3	1.5	2.3	4.7	3.2	5.9	4.4
250	1.3	1.4	1.8	3.1	2.5	3.8	3.1
125	1.3	1.3	1.6	2.5	2.3	2.9	2.6
62.5	1.3	1.3	1.5	2.3	2.0	2.3	2.3
31.2	1.3	1.3	1.3	1.6	1.7	1.9	2.0
15.6	1.3	1.3	1.3	1.4	1.6	1.8	1.8
7.8	1.3	1.3	1.3	1.3	1.3	1.4	1.5

Standard proteins were serially diluted and 10 µl samples were run on a 4%-20% gradient gel. The gel was washed 3 x 5 minutes with 200 ml of ultrapure water and stained with 20 ml of GelCode® Blue Stain Reagent for 1 hour. After the Water Wash Enhancement™ Step, the gel was scanned within the visible spectrum using a Bio-Rad Molecular Imager GS-700. The data (relative adsorption) were processed using Microsoft Excel and indicate that these proteins respond to the staining reagent at different linear ranges. The measurement of 1.3 is the background absorption.

Cited References

1. Chu, R. and Vigna, R.A. (1997). Water wash-enhanced protein staining with GelCode® Coomassie Blue Stain Reagent. *Previews* **1(4)**, 18-21.
2. Chu, R. and Vigna, R.A. (1998). SDS-PAGE gel staining with GelCode® Coomassie Blue Stain Reagent saves time and cost while delivering superior results. *Previews* **2(1)**, 10-11.
3. Sambrook, J., *et.al.* (1989). Molecular Cloning: A Laboratory Manual. p. 18.55.

Product References

Aulak, K.S., *et al.* (2001). Proteomic method identifies proteins nitrated in vivo during inflammatory challenge. *PNAS* **98**, 12056-61.

Hilton, J.M., *et al.* (2001). Phosphorylation of a synaptic vesicle-associated protein by an inositol hexakiphosphate-regulated protein kinase. *J. Biol. Chem.* **276**, 16341-7.

Lim, J., et al. (2002). Metastable macromolecular complexes containing high mobility group nucleosome-binding chromosomal proteins in HeLa nuclei. J. *Biol. Chem.* **277**, 20774-82.

Tani, M., *et al.* (2000). Purification and characterization of neutral ceramidase from mouse liver. *J. Biol. Chem.* **275**, 3462-8.

Mateer, S.C., *et al.* (2002). The mechanism for regulation of the F-actin binding activity of IQGAP1 by calcium/calmodulin. *J. Biol. Chem.* **277**, 2324-33.