INSTRUCTIONS

GelCode® Blue Stain Reagent

24590 24592

0714.4

Number	Description
24590	**GelCode Blue Stain Reagent**, 500mL, sufficient for 20 mini gels
24592	**GelCode Blue Stain Reagent**, 3.5L, sufficient for 175 mini gels

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

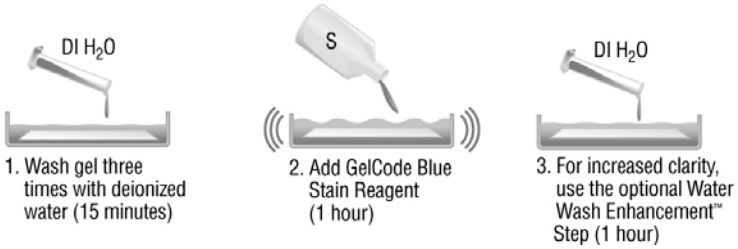
Storage: Upon receipt store product at 4°C. Product is stable up to 6 months at room temperature and up to 1 year at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific GelCode Blue Stain Reagent uses 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 step) further enhances staining sensitivity and yields a clear background.[1] With GelCode Blue Stain there is no need for multi-step destaining procedures typically 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.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Procedure for Staining Gels

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.

1. Wash SDS-PAGE or native PAGE gels as follows:
   - SDS-PAGE: After electrophoresis, place gel in a clean tray and rinse 3 × 5 minutes with 100-200mL of ultrapure water. Alternatively, wash gel in 1-2L of ultrapure water with gentle shaking for 15 minutes.
   - Native PAGE: Rinse gel with ultrapure water for 5 minutes.
2. 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.
3. Add 20mL of GelCode Blue Stain Reagent for an 8 × 10cm mini gel. Use more reagent as needed if using a large tray. 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.
4. To destain (water wash enhancement step) gel, replace Stain Reagent with ultrapure water. For optimal results, change water several times for 1-2 hours. This step enhances stain sensitivity, as weak protein bands continue to develop.

Procedure for Staining Membranes

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

   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.
2. Add 20mL of GelCode Blue Stain Reagent for an 8 × 10cm membrane. Use more reagent as needed if using a large tray. Incubate on an orbital shaker for 2-5 minutes.
3. 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 (12ng vs. 8ng for the standard protocol). Bands will develop in approximately 30 minutes using standard 1mm thick mini gels. Larger or thicker gels may require additional volumes of reagents and/or longer microwave and incubation times.

1. After electrophoresis, place gel into a microwavable tray containing 100mL of ultrapure water, and microwave for 90 seconds and discard water. Add 100mL of ultrapure water, and microwave again for 90 seconds and discard water. Add ultrapure water and place tray on an orbital shaker for 5 minutes.
2. 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.
3. Discard water wash from gel. Add 50mL 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.
4. To Destain (water wash enhancement step), discard staining reagent and replace with 200mL 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 > 1mm thick	Perform water wash enhancement step for 2-4 hours; alternatively, use thinner gels.

Related Thermo Scientific Products

26619 **PageRuler Plus Prestained Protein Ladder, 10-250kDa**

26623 **Spectra Multicolor Broad Range Protein Ladder**

24594 **GelCode Blue Safe Protein Stain,** 1L

24580 **Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes**

24612 **Pierce Silver Stain Kit**

24597 **Pierce Color Silver Stain Kit**

28378 **BupH™ Tris-Glycine-SDS Buffer Packs,** 40 packs

Additional Information

A. Visit our web site for additional information relating to this product including the following:

- GelCode Blue Stain Reagent FAQ
- Tech Tip #50: Process stained polyacrylamide gel pieces for mass spectrometry
- Tech Tip #51: Purify proteins from polyacrylamide gels

B. Performance Characteristics of GelCode Blue Stain Reagent

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

Protein (ng)	Myosin H-chain (200kD)	Phosphorylase b (97.4kD)	Bovine serum albumin (68kD)	Ovalbumin (43kD)	Carbonic anhydrase (29kD)	Lacto globulin (14.3kD)	Lysozyme (18.4kD)
3000	3.5	5.1	9.4	17.6	10.3	19.5	14.8
2000	2.7	3.8	7.3	14.2	8.4	16.0	11.8
1000	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

Proteins were serially diluted and 10µL samples were run on a 4%-20% gradient gel. The gel was washed 3 × 5 minutes with 200mL of ultrapure water and stained with 20mL 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 value 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.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.