Technical Review

Gel Stains

FEATURING...

GelCode® Blue Stain Reagent

GelCode® SilverSNAP™ Stain

GelCode® E-Zinc™ Reversible Stain Kits
Protein Staining

Introduction

Coomassie® Brilliant Blue is the most common dye used to stain proteins on polyacrylamide gels. The dye was originally developed as an acid wool dye, but found favor among life scientists because of its ease of use and relatively good sensitivity. Although alternative methods, such as silver staining, heavy metal staining and fluorescent dye stains have been developed, they are not as easy to use as Coomassie® stains. These methods have only been used for some specific applications.

Coomassie® staining requires an acidic environment to enhance the ionic interaction between the dyes and basic amino acids. Classically, the R-250 form of the dye is dissolved in a solution containing 20%-45% methanol and 5%-10% acetic acid at concentrations between 0.1%-0.25%. After the gel is stained, protein bands are not visible before destaining with methanol/acetic acid to remove the characteristic background staining. The main drawback to traditional Coomassie® Brilliant Blue staining methods is the long time period required for staining and destaining. Overnight destaining periods are usually necessary to achieve a clear background. It is common that the staining intensity of protein bands is decreased by prolonged destaining periods, resulting in reduced sensitivity. Using this method, a gel typically cannot be analyzed until the day after it is run.

The G-250 form of Coomassie® Brilliant Blue is typically used as a colloidal suspension. The colloidal particles do not penetrate the gel, but the dye molecules are extracted from the colloid by the proteins in the gels. The proteins become tinted by the dye molecules and, because there is little coloration in the bulk of the gel, destaining is not required. A method developed by Neuhoff, V., et al. has improved the sensitivity of Coomassie® Brilliant Blue G-250 stain to the nanogram level. The main drawbacks of this method is that it requires long (12-hour) staining times to reach a maximum sensitivity, and the band signal-to-noise ratio is low.

Pierce recently developed a Coomassie® Brilliant Blue G-250-based protein stain, GelCode® Blue Stain Reagent, which uses the colloidal properties of Coomassie® G-250 for protein staining in polyacrylamide gels. This exclusive Pierce formulation yields staining properties unique to a Coomassie® Brilliant Blue G-based protein stain. For example, only the protein is stained, not the gel, allowing protein bands to be viewed directly within the staining reagent during the staining process. In addition, the staining sensitivity reaches its maximum within one hour. Longer (overnight) staining does not increase the background. In addition, a simple water wash after staining significantly increases the staining sensitivity, and the gel background is crystal-clear without having to destain with methanol/acetic acid.

Materials and Methods

I) Protein Electrophoresis

Protein molecular weight standards consisting of myosin H-chain (200 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (68 kDa), ovalbumin...
(43 kDa), carbonic anhydrase (29 kDa), β-lactoglobulin (18.4 kDa) and lysozyme (14.3 kDa) were reconstituted with deionized water at an approximate concentration of 1 mg/ml of each protein. The reconstituted proteins were further diluted serially in 1X SDS-PAGE buffer (50 mM Tris•HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol) and boiled for 5 minutes to denature the protein samples. Samples were loaded identically onto two 4%-20% gradient, 8 cm x 7.5 cm mini gels, and run with constant current at 20 mA for each gel until the bromophenol blue dye reaches the bottom.

II) Protein Staining
After electrophoresis, one of the gels was rinsed with 200 ml of deionized water, 3 times for 5 minutes and then stained with 20 ml of GelCode® Blue Stain Reagent for 1 hour. After staining, the staining reagent was replaced with 200 ml of deionized water. A gentle agitation was used during the entire process to ensure that the gel was treated uniformly. The other gel was stained with a commercial staining reagent containing 0.25% Coomassie® Blue G-250, 25% methanol and 5% acetic acid for one hour and destained with several changes of 25% methanol and 5% acetic acid.

III) Staining of Proteins After Western Blot Transfer
Approximately 50 µg of bacterial E. coli cell lysates expressing polyhistidine-tagged (PHT) mouse dihydrofolate reductase and baculovirus polyhedrin protein respectively, were lysed with SDS-PAGE loading buffer (1X) and boiled for 5 minutes before loading onto two 4%-20% gradient mini gels. After electrophoresis, one of the gels was stained with GelCode® Blue Stain Reagent as previously described, and the other gel was transferred with a semi-dry transfer unit for 1 hour at a current of 0.8 mA/cm². After transfer, the membrane was processed for detection of PHT proteins using INDIA™ HisProbe™-HRP* (Product # 15165) following the manufacturer’s instructions. The transferred gel was rinsed once with deionized water for 20 seconds and stained with GelCode® Blue Stain Reagent for one hour, followed by equilibration in water.

Results
Protein Bands Visible Without Destaining
Colloidal staining of proteins with GelCode® Blue Stain Reagent following PAGE allows highly sensitive and background-free staining. Standard proteins were serially diluted, run in 4%-20% SDS-PAGE gels and washed three times with deionized water before staining with GelCode® Blue Stain Reagent. The colloidal dye stains protein rapidly; protein bands at concentration above 1 µg were visible within only a few minutes in the staining reagent. With one-hour staining, the staining intensity of protein bands reaches a maximum and standard proteins at concentrations above 250 ng were clearly visible (Figure 1A). Unlike other staining systems, the GelCode® Blue Stain Reagent only stains protein; the bulk of the gel remains virtually clear. The background staining did not increase even with an overnight staining (data not shown).

Water Wash Enhancement™ Step
After a one-hour staining, the staining reagent was replaced with deionized water. The staining sensitivity was significantly increased after placing the gel in deionized water for a period of 1-2 hours. On the water-equilibrated gel, standard protein bands at a concentration of 31 ng were clearly visible. Some of these standard proteins were even visible at concentrations as low as 8 ng (Figure 1B). Variations were observed among different standard proteins. This result could be due to discrepancy in
the amount of each individual protein in the standard or because individual proteins interact with the dye differently. In contrast, no protein bands were visible on the gel stained with the commercial staining reagent for one hour (Figure 1C) before destaining, and the gel retained very high background even after destaining with 25% methanol/5% acetic acid for 1 hour (Figure 1D).

Figure 1. Comparison of GelCode® Blue Stain Reagent With a Classical Coomassie® Stain Formulation. Standard proteins were serially diluted and run on two 4%-20% gradient gels. One gel was stained with GelCode® Blue Stain Reagent for one hour (A), and then washed with deionized water for one hour (B). The other gel was stained with 0.25% Coomassie® Blue G-250 in 25% methanol and 5% acetic acid for one hour (C), and then destained with 25% methanol and 5% acetic acid for one hour (D). The amount of each standard protein, as described in the text, was approximately 3,000 ng, 2,000 ng, 1,000 ng, 500 ng, 250 ng, 125 ng, 62 ng, 31 ng, 16 ng and 8 ng, in each lane (left to right).

### Linear Range Staining

Scanning the visible spectrum of the stained gel using a Bio-Rad Molecular Imager® GS-700 indicated that most of the standard proteins, such as bovine serum albumin, ovalbumin, carbonic anhydrase, lactoglobulin and lysozyme, responded to the staining reagent at a linear range between 61 ng to 2,000 ng per band, while myosin H-chain and phosphorylase b exhibited a linear range between 500 ng to 3,000 ng (Figure 2).

**A.** Myosin H-chain (200 kDa)  
**B.** Phosphorylase b (94.7 kDa)  
**C.** Bovine serum albumin (68 kDa)  
**D.** Ovalbumin (43 kDa)  
**E.** Carbonic anhydrase (29 kDa)  
**F.** Lactoglobulin (18.4 kDa)  
**G.** Lysozyme (14.3 kDa)

Figure 2. The Linear Range of Standard Proteins Stained With GelCode® Blue Stain Reagent. The gels shown in Figure 1B were scanned within the visible spectrum using a Bio-Rad Molecular Imager® GS-700. The data was processed with Microsoft Excel and converted to graphs. Figure 2B is the inset of Figure 2A at concentrations between 8 ng to 250 ng.

### Convenient Protein Staining After Western Blot Transfer

It has been observed that high concentrations of SDS in the PAGE gel interfere with the GelCode® Blue Stain Reagent. A simple water wash is usually sufficient to remove SDS from the gel, achieving excellent results. For Western Blot-transferred gels, most of the SDS has been removed during transfer. Therefore, this washing step can be simplified to a...
single rinse with deionized water. Protein bands on a transferred gel are developed more rapidly than protein bands on untransferred gel during the staining process. Following a one-hour staining and a one-hour water equilibration, trace amounts of proteins remaining on the gel can be easily detected (Figure 3). This feature is particularly useful for evaluating protein loading and for checking if proteins on SDS-PAGE gels are uniformly and completely transferred.

**Conclusion**

As demonstrated by our results, the GelCode® Blue Stain Reagent represents a new generation of Coomassie® Blue Stain with several unique properties: (1) easily viewed protein bands during the staining process, (2) elimination of the methanol/acetic acid destaining step and (3) enhancement of staining sensitivity by a simple water wash step. Other benefits of GelCode® Blue Stain Reagent include a crystal-clear gel background, no gel shrinkage, significantly shortened timeline to a result, time-savings and reduced costs.

**REFERENCES**


**Ordering Information**

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<td>GelCode® Blue Stain Reagent</td>
<td>500 ml Sufficient for staining up to 25 SDS-PAGE 8 cm x 10 cm mini gels</td>
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Coomassie® is a registered trademark of ICI Americas.

*Patent Pending*
Rapid and Ultrasensitive PAGE Gel Stains:

Introducing GelCode® SilverSNAP™ Stain and GelCode® E-Zinc™ Reversible Stain Kits

Coomassie® stain, silver stain and heavy metal (Zn) stain are often used for protein detection in polyacrylamide gels following electrophoresis. Although Coomassie® is the dye most frequently used for protein staining in polyacrylamide gels, silver staining has a tendency to be much more sensitive. The main disadvantage associated with common Coomassie® stains is that they require a time-consuming and foul-smelling methanol-acetic acid destaining procedure. GelCode® Blue Stain Reagent, a new Coomassie® stain developed by Pierce Chemical Company, does not require cumbersome destaining steps. The reagent stains only protein, not the gel, resulting in a crystal-clear background after a Water Wash Enhancement™ Step. Thus GelCode® Blue Stain Reagent is the ideal stain for many applications. In addition to GelCode® Blue Stain Reagent, Pierce recently developed two new related kits: GelCode® SilverSNAP™ and GelCode® E-Zinc™ Reversible Stains.

GelCode® SilverSNAP™ Stain Kit Provides Superior Sensitivity

The lower detection limit of GelCode® Blue Stain Reagent is 8-10 ng, which is sufficient for most applications. In some applications such as two-dimensional (2-D) PAGE gel staining, however, a higher sensitivity is required. The GelCode® SilverSNAP™ Stain Kit (Product # 24602) provides greater sensitivity than Coomassie® stain kits. The kit consists of three ready-to-use components and a simple procedure that can be completed within 90 minutes. As shown in Figure 1, as little as 0.25 ng of protein molecular weight standards separated by a 4%-20% SDS-PAGE gel are clearly visible. The yellowish background associated with common stains is eliminated.

Three GelCode® Stain Products are Complementary

GelCode® Blue Stain Reagent, GelCode® E-Zinc™ Reversible Stain and GelCode® SilverSNAP™ Stain are designed for different applications. Table 1 shows each stain’s characteristics. No matter what your application, one of these stains will deliver satisfactory results.

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Table 1. Comparison of Three GelCode® Stain Products
silver stain is eliminated with the GelCode® SilverSNAP™ Stain. In comparative experiments, some proteins that were not detectable by the Silver Stain from Vendor B are clearly visible with GelCode® SilverSNAP™ Stain. Thus the GelCode® SilverSNAP™ Stain Kit is more reliable. This kit has also been tested for DNA and RNA staining on PAGE gels.

GelCode® E-Zinc™ Reversible Stain Kit is Ideal for Recovering Proteins From PAGE Gels and Western Blots After Staining

The length of time required to use Coomassie® stains and silver stains is sometimes cited as a drawback of both techniques. In addition, once the protein is stained, it is difficult to remove the dye from the protein-dye complex. The dye-contaminated protein is not appropriate for downstream applications, such as antibody generation and/or immunological detection. As shown in Figure 2, GelCode® E-Zinc™ Reversible Stain Kit (Product # 24582) is ideal for rapid detection of proteins on a PAGE gel with a sensitivity equivalent to silver stain.

Figure 1. Comparison of GelCode® SilverSNAP™ Stain With a Silver Stain From Vendor B. Protein molecular weight standards, as indicated at the top of the gel, were separated on 15-well pre-cast 4%-20% SDS-PAGE gels. For GelCode® SilverSNAP™ Stain (top), following fixing and washing, the gel was stained with SilverSNAP™ Stain for 30 minutes, developed with SilverSNAP™ Developer for approximately 5 minutes, and stopped with 5% acetic acid. For Silver Stain (Vendor B) (bottom), the vendor’s procedure was followed. Note that GelCode® SilverSNAP™ Stain not only provides higher sensitivity, but also stains all proteins. Because all GelCode® SilverSNAP™ Stain Kit components are ready-to-use, the background caused by mixing multiple components – as seen when the vendor B stain is used – is eliminated.

Figure 2. Comparison of GelCode® E-Zinc™ Reversible Stain With Zn Stain (Vendor B), GelCode® Blue Stain Reagent and GelCode® SilverSNAP™ Stain. Protein molecular weight standards, the same as those used in Figure 1, were separated on 15-well pre-cast 4%-20% SDS-PAGE gels. For Zn stain, the gels were stained with the stain solution for 10 minutes and developed with the developer solution for approximately 2 minutes. GelCode® SilverSNAP™ Stain was used as indicated in Figure 1. GelCode® Blue Stain Reagent was used as described earlier.
No matter what your application, one of these stains will deliver satisfactory results.

for rapid detection of proteins on a PAGE gel with a sensitivity equivalent to silver stain. The two-step procedure takes less than 15 minutes and the stain can be removed completely by the E-Zinc™ Eraser. For example, Figure 3 demonstrates the use of the GelCode® E-Zinc™ Reversible Stain Kit for the purification of a recombinant protein from PAGE gel.

By using the reversible stain, the final protein preparation is free of dye contamination and is ideal for antibody generation. In addition to reversibility, GelCode® E-Zinc™ Reversible Stain may also be used for rapid analysis of purified protein and the composition of protein components in a diluted sample on PAGE gels.

**Figure 3.** Purification of Inclusion Body Protein From PAGE Gel After GelCode® E-Zinc™ Reversible Stain. The inclusion body of a GST fusion protein expressed in *E. coli* was extracted and purified by B-PER® Bacterial Protein Extraction Reagent (Product # 78248). As some of the bacterial proteins are associated with the GST-TSP recombinant protein within the inclusion body, the inclusion body preparation was further purified through an SDS-PAGE gel to obtain homogeneous recombinant protein. On a preparative SDS-PAGE gel, 4 mg of crude GST-TSP inclusion body was dissolved in 1X SDS-PAGE sample buffer, loaded and separated from contaminants. The preparative gel was stained with GelCode® E-Zinc™ Reversible Stain Kit and the fusion protein band was excised. After removing Zn by destaining with the EZ-Zinc™ Stain Eraser, the GST fusion protein was electro-eluted from the gel and the purity was analyzed by loading various amounts of the eluted sample on an SDS-PAGE mini gel stained with GelCode® Blue Stain Reagent. **Lane 1:** uninduced total bacterial protein; **Lane 2:** induced bacterial protein; **Lane 3:** the soluble protein extracted by B-PER® Reagent; **Lanes 4-6:** two washes with 1:10 diluted B-PER® Reagent; **Lane 6:** purified inclusion body protein; and **Lanes 7-9:** 1, 2 and 4 µg of GST fusion protein electro-eluted from PAGE gel after Zn stain. Note that the insoluble GST fusion protein seen in Lane 2 is not present in Lane 3.

**Recommended reading or information on the use of GelCode® Blue Stain Reagent for zymogram staining:**


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Coomassie® is a registered trademark of ICI Americas. PhastGel® is a registered trademark of Pharmacia LKB Biotechnology AB.
Compatible Products

Gel Forming Reagents, Markers and Staining Kits

Ammonium Persulfate

A useful catalyst for acrylamide gel polymerization.

\[(\text{NH}_4\text{H}_2\text{S}_2\text{O}_8)\]

Ammonium Persulfate

M.W. 228.20

ImmunoPure® Lane Marker Sample Buffers

Ready-to-use lane markers that can be used for Western blots!

Features/Benefits:
- Bright pink hydrophobic tracking dye for SDS-PAGE
- Ability to see proper transfer of proteins from gel to nitrocellulose membrane
- Easy cutting of sample lanes when different antibody systems are used in the “shaker” method
- Dye front is easily shown on both the gel and the nitrocellulose membrane for determination of molecular weight (Rf values)
- Easy alignment of sample lanes containing transferred protein in the Pierce Fast Blot-Developer™ Systems
- DTT is used in place of β-Mercaptoethanol in the Reducing Sample Buffer, so strong odors and auto-oxidation are avoided

2-Mercaptoethanol (2-ME)

Reduces dithiol bonds in peptides and proteins.

\[
\text{HS} \xrightarrow{\text{OH}}
\]

2-Mercaptoethanol

M.W. 78.13

SDS

Useful for resolving viral proteins during gel electrophoresis and for when renaturation after SDS-PAGE is required.

\[
\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}
\]

SDS

M.W. 288.38

Typical Alkyl Chain Length Distribution for Pierce SDS Lauryl Grade

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**SDS C₁₂, Sequanal Grade**

Contains very low levels of C₁₆ alkyl sulfate.

Specifications:
- White powder
- Greater than 99% alkyl sulfate
- Greater than 98% C₁₂ alkyl sulfate
- 10% solution is clear, colorless and free of particulate matter

**Specifications:**
- Purity: > 99.9%
- Refractive Index: 1.417-1.419
- Boiling Range: 119-121˚C

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*See the Detergents section for more information on SDS.*

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**TEMED**

Greater than 99% pure!

\[ C₆H₁₆N₂ \]

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**Specifications:**
- Purity: > 99.9%
- Refractive Index: 1.417-1.419
- Boiling Range: 119-121˚C

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**Urea (Sequanal Grade)**

A low UV-absorbing protein denaturant.

\[ \text{H}_₂\text{N}^\cdot\text{NH}_₂ \]

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**Specifications:**
- \( A_{280} \) nm: < 0.100

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**Typical Alkyl Chain Length Distribution for Pierce C₁₂ Sequanal Grade**

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**General Electrophoresis References**
