PROMETHEUS™
Protein Biology Products

Instruction Manual

Coomassie Brilliant Blue G-250 Protein Stain — Catalog No.: 18-446 and 18-447

Introduction:

Polyacrylamide gel electrophoresis is one of the most widely used methods to analyze proteins and after separation by electrophoresis, protein bands need to be detected with a suitable protein stain. Our Coomassie Brilliant Blue G-250 Protein Stain is a ready to use protein staining solution. The Coomassie G-250 dye binds to proteins through ionic interactions between the sulfonic acid groups of the dye and positive protein amine groups and through Van der Waals attractions. The capability of G-250 to create a rapid and convenient staining procedure is due to its particular properties and manifests a leuco form below pH 2. Solutions of the dye, dark blue black at pH 7, turn a clear tan upon acidification. The leuco form recovers its blue color upon binding to protein, apparently due to the more neutral pH of the environment around the protein molecule. Under ideal staining conditions, a gel placed in Coomassie Brilliant Blue G-250 Stain will manifest blue protein bands on a light amber background. The protein bands develop rapidly and there is no need to de-stain, as the background color is not dark. Our stain can be used with SDS-PAGE, Native PAGE, Tricine gels with fixation step, 2-D Electrophoresis, etc. It stains the proteins with high band visibility and the protein bands up to 0.1 µg protein can be observed within 5 minutes, while staining the gel. The sensitivity of our Coomassie Brilliant Blue G-250 Protein Stain is up to 8 ng protein/ band, as observed in 4-20% SDS-PAGE gel loaded with BSA.

Features:

- Ready to use solution
- Fast protein staining - in about 60-90 minutes
- Detects up to 8 ng protein per band
- Protein bands appear and visible while the gel in stain
- Compatible with fixing by alcohol
- Mass Spec compatible
- Non-hazardous solution

Item(s) Supplied

Catalog No.	Name	Size	Storage Temp
10491	Coomassie Brilliant Blue G-250 Protein Stain	1 L	18 to 26°C
10491-1	Coomassie Brilliant Blue G-250 Protein Stain	1 Gal	18 to 26°C

Staining Protocols:

A. For SDS-PAGE Mini Gels

NOTE: This protocol is written for mini gels (8-10") and if large size gels (16-20"), double the volumes used in the protocol.

1. After the completion of electrophoresis step, remove the gel and place it in a smooth plastic tray, then add about 200 ml of deionized (DI) water into it.
2. Wash the gel 3 times in about 200 ml DI Water, 5 minutes each time on a rotary shaker to remove the SDS present in the running buffer. NOTE: If the gel is not thoroughly washed, the presence of SDS in it will interfere with the protein staining and band intensity.
3. Remove all water from the container to minimize the dilution of the Coomassie G-250 stain
4. Add 50 ml of Coomassie Brilliant Blue G-250 Stain to cover the gel (sufficient for a mini gels). Put the container with gel in stain on an orbital shaker and gently shake it for 1 hour at room temperature.
5. Protein bands will start appearing and most of them visible within 15-20 minutes with maximum intensity in 1 hour.
6. Pour off the stain carefully and rinse the gel with 200 ml DI water for 30 minutes to overnight, the bands will become prominent and sharp. Rinsing the gel thoroughly in DI water will reduce the background.
7. Proceed with downstream applications. Stain gels can be stored in water for 2-3 days.

B. For Peptide Gels

Fix the gel in 40% methanol, 10% acetic acid for 30 minutes, remove all free fixing agent and follow the above protocol used for SDS-PAGE starting from step 4 onward and increase the time at step 4 above to 2 hours.

RELATED PRODUCTS:

1. **Protein Extraction Buffers/ Kits (Cat. No. 18-400, 18-402, 18-404, 18-406, 18-409 and 18-411)**
   For extracting proteins from Bacteria, Insects Cells, Mammalian cells, Tissues and Yeast samples

2. **Protease Inhibitor Cocktails (Cat. No. 18-420, 18-425, 18-427, 18-430, 18-433 and 18-436)**
   For inhibiting protease activity in the protein extracts: General, Bacterial, Mammalian, Plant, Recombinant and Yeast/Fungi.