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A Geno Technology, Inc. (USA) brand name

# Colloidal Blue Stain

## A Protein Gel Stain
## Based on Colloidal Coomassie Blue G250

## (Cat. # 786-500)

## INTRODUCTION

G-Biosciences Colloidal Blue Stain is a mass spectrometry compatible stain that offers nanogram sensitive detection of proteins. The stain offers ten times more sensitivity compared to classical Coomassie R- or G-250 detection. The Colloidal Blue Stain is able to detect <10ng BSA. In addition, the protein bands are visualized on crystal clear backgrounds, following a water wash, allowing for optimal densitometry results.

Based on the original work of Volker Neuhoff et al[1]. Using the colloidal properties of G-250 that reduces free dye in solution, they demonstrated shorter staining times, high sensitivity, clear background without the need for destaining or stepwise staining.

G-Biosciences Colloidal Blue Stain is a single, ready-to-use reagent. Sufficient reagent is supplied for 25 mini gels.

## ITEM(S) SUPPLIED

| Description | Size |
|---|---|
| Colloidal Blue Stain | 1L |

## STORAGE CONDITIONS

The Colloidal Blue Stain is shipped at ambient temperature. Upon receipt, store at room temperature. The stain is stable for 1 year when stored at room temperature.

## ADDITIONAL ITEM(S) REQUIRED

- Deionized water
- Methanol
- 40% Methanol, 10% Acetic acid for fixing peptides and IEF strips
- 25% Ammonium sulfate solution or gel drying reagents for long term storage

## PROTOCOL

***Polyacrylamide Gel Staining Protocol***

1. Wash polyacrylamide gel 3 times for 5 minutes in a large volume of deionized water.
2. Remove all free water from the gel.
3. Mix the Colloidal Blue Stain, by inverting the bottle for 30 seconds, to uniformly suspend the Colloidal Blue Stain.
   ***NOTE:*** *The Colloidal Blue Stain bottle contains mixing beads to aid in mixing.*
4. Prepare a suitable volume of stain to cover the gel by combining 4 parts Colloidal Blue Stain with 1 part methanol. For mini gels, mix 40ml Colloidal Blue Stain with 10 ml methanol.
5. Immediately, add the stain to cover the gel and gently shake the gel for 1-2 hour maximum. Protein bands may be visible within 5-10 minutes and reach a maximum intensity within 1 hour in most applications. Incubation longer than 1 hour has marginal impact on staining and will not increase the background.
6. Rinse the stained gel in a large volume of deionized water, 3 times for 10-15 minutes each.
7. The stained gels can be stored in water for up to 3 days. For longer storage, either dry the gel (Gel Drying Solution, Cat. # 786-685) or store in 25% ammonium sulfate solution at room temperature.

***Peptide Gel Staining Protocol for Mass Spectrometry***

*For mass spectrometry analysis of peptides, follow this procedure for staining peptides (<20kDa) on all gel types and for peptides and proteins in Bis-Tris gels.*

1. Fix the gel in 40% methanol, 10% acetic acid for 30 minutes.
2. Remove the free fixing agent and continue with Step 3 in the protocol above.
3. After staining wash the gel with water for 2 hours

***Isoelectric Focusing (IEF) Gel Staining Protocol***

1. Fix the gel in 12% TCA (w/v) with 3.5% sulfosalicylic acid (w/v) or 40% methanol, 10% acetic acid for 30 minutes.
2. Remove the free fixing agent and continue with Step 1 in the polyacrylamide gel staining protocol above.

## TROUBLESHOOTING

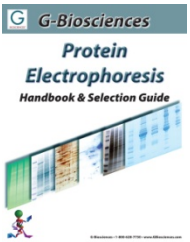
| Issue | Possible Reason | Suggested Solution |
|---|---|---|
| No Protein Bands Visible | The gel was not adequately washed with water | Add fresh stain and stain for 1-2 hrs |
| | Water was not completely removed from the gel after washing step | |
| | Not enough stain was used for staining the gel | |
| Poor staining of proteins | Inadequate washing, water was not removed completely or not enough stain for staining the gel | Add fresh stain and stain for 1-2 hrs |
| | | After staining extend the washing step to 1-2 hours. |
| | Gel matrix density and gel buffer composition reduced the diffusion of stain across the gel matrix to react with the proteins | Add fresh stain and increase the incubation time to 2 hours or longer |
| | | After staining extend the washing step to 1-2 hours. |
| | Protein diffused in the gel, poor gel quality, not enough protein present in the sample, protein loss during sample loading step or poor handling. | Repeat the electrophoresis with either a different gel or increase the amount of protein loaded on the gel. |
| | | After staining extend the washing step to 1-2 hours |
| The gel has high background | Inadequate washing, poor handling, poor quality of water or residual SDS contamination in the gel | Extended washing with deionized water will remove the background staining |

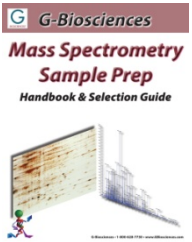
## REFERENCES

1. Neuhoff, V. et al (1988) Electrophoresis, 9:255-62

## RELATED PRODUCTS

Download our Protein Electrophoresis and Mass Spectrometry Sample Preparation Handbooks.

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