
CooBlue Stain for Proteins in Gel

Product Description

Name: **CooBlue Stain for Proteins in Gel**
(1X Ready-to-use Colloidal Coomassie Stain for Proteins in Gel)
Optimized for both analytical and **extraction** applications

Cat. Number: UP47255A, 500 mL UP47255B, 4.5 L

- Hands-Off! Stain and read directly (no destaining)
- High Sensitivity, below 10-20 ng of Protein per Band
- Slight/mild fixation to proteins: no denaturant, keep proteins in native form
- Compatible with further analysis of gel (silver staining) or proteins (MALDI TOF)
- Compatible with protein extraction (electroelution)
- Safe and environment friendly: No More Methanol and Acetic Acid !

Applications:

- Protein staining to identify desired proteins before electro elution with high recovery yield /purification/extraction, and that is compatible with further Maldi MS analysis or sequencing
- Protein staining for control the efficiency of protein transfer / blotting
- analytical proteomics (see note below)

Storage: +4°C, stable for 1 year when stored properly (L)

Introduction

Coomassie® Blue staining of proteins in SDS-PAGE gels is a daily procedure in many laboratories. It is popular among life science researchers, due to its good sensitivity and relative ease of use. Traditionally, Coomassie Blue staining requires a methanol and acetic acid solution to achieve staining and destaining. The risk increases with contact of hazardous exposure and this product produces an unpleasant pungent odor.

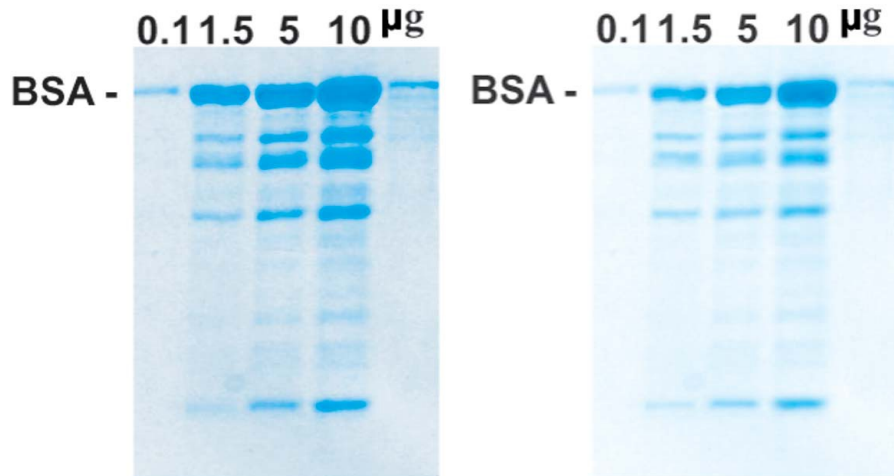
CooBlue Stain is a convenient alternative to traditional Coomassie Blue staining procedures, based on a colloidal formulation. Environmentally friendly, this ready-to-use stain does not contain methanol and acetic acid and does not require hazardous solvents for destaining. The protein bands are visible directly during the staining process. After staining, a simple and quick washing with water yields clear background, allowing optimal sensitivity. There is no need for multistep destainings as required by conventional coomassie stainings. The simple "hands-off" staining/destaining procedure saves valuable time while reducing the handling of hazardous materials and solvent waste in your laboratory.

CooBlue Staining exhibits high sensitivity, below 20 ng of protein per band, and up to 8-10ng depending on conditions of use (fixed gel or not, protein nature...), making it ideal for most applications including SDS-PAGE and 2D gels.

For only analytical applications, we recommend using our CooBlueFX Instant stain #UPG4562A or CooBlueMAX Instant stain #R2034A, that combine gel fixation and gel staining and get more intense and stable staining (but not reversible).

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CooBlue. Protein Band are easily visualized without wash (left), and after a simple quick wash (right) with lowered background.



Directions for Use

CooBlue Staining Procedure for SDS-PAGE:

Note: This protocol is optimised for 8 x 10 cm minigels 10% SDS-PAGE (1 mm thick). Further notes and comments give guideline for optimizations and other applications.

- Prepare the gel: Remove the stacking gel from the separating gel.
 - The gel can be fixed previously or not for direct staining. Non fixed gels allow further applications like recovery of proteins by electroelution. However, the colloidal CooBlue stain penetrates better if the gel is fixed. Thus fixed gels are preferred for greater sensitivity.
 - Fixation may be performed after a rapid water wash with (30% methanol, 10% acetic acid) for 30minutes.
 - If MOPS or MES containing buffer is used, fix the gel first with 50% Methanol 7% acetic acid for 15min then wash.
 - Pre-Wash with Deionized Water, 15 min for denaturing gels with gentle shaking.
- For optimal results, use 300 ml of ultra-pure water 3 x 5 minutes per minigels. SDS interfere staining procedure; so we recommend to wash longer if the thickness or acrylamide percentage is important >15% (see the table below)
- Note: For native gels, a simple 5min pre-wash is usually sufficient

- Mix gently the CooBlue stain before use.
- Note: Close the vial immediately after use and store at +4°C.

- Stain for 1 hour with CooBlue Stain: submerge the gel completely with 20ml per minigel 8x10cm² (more may be needed depending on the tray), and gently shake. Stain intensity reaches a maximum within approximately 1 hour. Gels may be stained overnight without increased background.

- Quick wash the gel with ultrapure water for immediate reading, or 3x10 min. Destain in deionized water for optimal result

Note: The gel or pieces can be destained overnight or more for some applications (other detections, extraction of proteins)

Comments for optimizations, and other applications:

-Longer incubations are needed for **thicker or higher-percentage of acrylamide in gels**.

Time Table for removing SDS from gels	Gel percentage	Time for shaking in water
	Protein gel <14%	10 minutes X 3 in 300 ml water
	Protein gel 14%-16%	30 minutes X 3 in 300 ml water
	Protein gel >16%	60 minutes X 3 in 300 ml water

Note: fixation may also be performed (see below)

- native gels electrophoresis**: the procedure depends on the application:
 - .If this is for an analytical application, one could perform a pre-fixation (see below).
 - .if it is for an extraction application, no fixation is required: proceed as for SDS-PAGE, but pre-wash may be reduced to 5 minutes. CooBlue guarantees protein staining in polyacrylamide gels with only slight fixation of the protein to the gel, allowing electro-elution of a desired protein from polyacrylamide gels with high recovery yield.

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-gels/buffers containing MOPS, MES: one should fix the gel to remove these interfering substances.

-**Fixation of the gel:** this step increases the sensitivity and the stability of staining, but preclude extraction applications. Incubate the gel under gentle skaking in a 50% methanol and 7% acetic acid solution for 15 minutes and then washed with ultrapure water.

-CooBlue (non fixed gels) is normally efficiently removed from protein by **electroelution**, dialysis and/or concentration making is compatible with further MS analysis.

-Troubleshooting

Problem	Cause	Solution
No band development	Gel is > 1 mm thick	Longer staining incubation
	Gel has high polyacrylamide concentration	Longer staining incubation
	SDS interference	Wash the gel with plenty of water before staining
Bands are faint	Destaining occurred at last step	Reduce washing time before reading Choose CooBlueFX UPG4562.
	Lack of sensitivity / protein load	Increase protein load
Undesired bands	Protein contamination	Check gel / buffers preparation
High background	Protein contamination	Check gel / buffers preparation
Staining not homogenous	Bubbles, uncovered edges...	Check for incubation conditions, increase washing and staining volumes

Quick Microwave Procedure:

This quick procedure (only 10-15min) uses a microwave to speed the staining, while preserving sensitivity (10 ng BSA). It is optimized for 1.0mm mini-gels. (*For 1.5 mm mini-gels, use the values in italic*)

Caution: Use caution using the stain in a microwave oven. Do not overheat the staining solutions.

1. After electrophoresis, place the gel in 100 ml of ultra pure water in a loosely covered container and microwave on High (950 to 1100 watts) for 1 minute until the solution almost boils.
2. Shake the gel on an orbital shaker for 1 minute (*2 minutes*). Discard the water.
3. Repeat Steps 1 and 2 two more times.
4. After the last wash, add 20 ml (*30 ml*) of CooBlue FX and microwave on High for 45 seconds to 1 minute (*1.5 minutes*) until the solution almost boils.
5. Shake the gel on an orbital shaker for 5 minutes (*10 minutes*).
6. Wash the gel in 100 ml of ultra pure water for 10 minutes on a shaker.

Related products:

See [BioSciences Innovations catalogue](#) and [e-search tool](#).

- High quality reagents for electrophoresis (acrylamides, buffers...). Inquire. [p.B194-B204](#)
- CooBlue FX Protein Gel stain [#UPG4562A](#): an analytical version (no fixation step, permanent staining) – and safe
- CooBlue MAX Protein Gel stain [#R3034A](#): an analytical version (no fixation step, permanent staining) with improved staining
- ProSave 5min Protein gel stain [#BP7121](#): combines high sensitivity & max. flexibility (analytical, compatible with MS, elution, WB...)
- LavaPurple [#67433A](#): the highest sensitive protein gel stain (50ng protein) , by fluorescence (MS compatible).

Ordering information

Further package sizes and pricing may be found at <http://www.interchim.com>

Please inquire for bulk quantities (availability, shipment conditions, etc).

Any questions please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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