FT-47255A

CooBlue Protein Gel Stain

Product Description

Name:	CooBlue Stain for Proteins in Gel Ideal for both analytical and extraction applications
Catalog Number:	**UP47255A**, 500mL (1X Ready-to-use Colloidal Coomassie Stain for Proteins in Gel) **UP47255B**, 4.5L (1X Ready-to-use Colloidal Coomassie Stain for Proteins in Gel) **CE2070**, 100ml 66X (makes 6.6L) (66X Concentrate: 100 ml Concentrated SeeBand and 165 ml Enhancing Buffer)

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

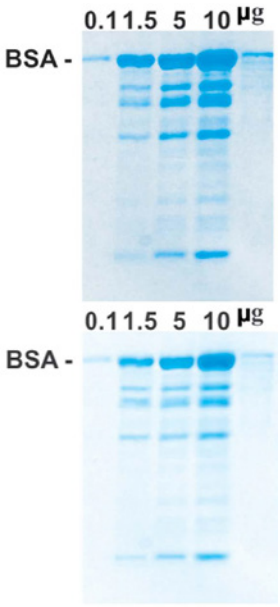
Storage

+4°C, stable for long term for +1 year. Shipped at RT. DO NOT FREEZE. (H)
Can be stored at Room Temperature.

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, acetic acid and phosphoric acid to fix, stain and destain, causing irritating and pungent odor, hazardous risks, and disposal problems. Additionaly, the fixation impeach recovery of proteins from gel, i.e. by electro-elution, and further use (i.e. analysis by MS or sequencing).

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 multi-step 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 SDS-PAGE, IEF and 2D gels.



NOTE: **For *only* analytical applications**, we recommend using our or the CooBlue FX-NPW #1B810, that combines gel fixation,(without need of pre-wash) and gel staining to get more intense and stable staining (staining is however less reversible).

Directions for Use

CooBlue Staining Procedure for SDS-PAGE gels:

Before starting: allow the CooBlue FX protein staining solution to reach RT under continuous agitation.
Note: This protocol is optimised for 8 x 10 cm2 minigels 10%
PAGE (1 mm thick).

- **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. The colloidal CooBlue stain penetrates better if the gel is fixed (CooBlue FX #UPG4562A may then be preferred).
  (optional) : fixation:
  - Fixation may be performed after a rapid water wash with (30% methanol, 10% acetic acid) for 30minutes. Discard soln.
  - If MOPS or MES containing buffer is used, fix the gel first with 50% Methanol 7% acetic acid for 15min then wash.
- **Wash** denaturing gels with Deionized Water, for 15 min with gentle shaking.
  Notes: SDS interferes with the staining procedure; so we recommend for optimal results, -to use 300 ml of ultra-pure water 3 x 5-10 minutes per minigels. -to wash longer if the thickness of gel (>2mm) or acrylamide percentage ratio (>15%) are important (see below 'Optimize') -for native gels (no SDS), a simple 5min pre-wash is usually sufficient.

- **Stain** for 1 hour with CooBlue Stain: Submerge the gel completely with CooBlue reagent. Gently shake tray.
  Notes: The CooBlue stain should be well temperature-equilibrated / mixed before use for optimal performance. Close the 500ml vial immediately after use.
  It can be store at room temperature when used daily or weekly.
  The reagent can be conveniently dispensed using appropriate pump #T34711 / CooBlue in 4.5L container.
  Use 20ml of CooBlue 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.

- **Read the gel:** Quick wash the gel with ultrapure water for immediate reading. Note : The Gel can be read also after just discarding the stain buffer, but a quick wash is easy to do and better.
  (optional) Destain for 3x10 min in deionized water for optimal reading (clears the gel and enhances stain sensitivity)
  Notes: Ultra fast washing can be achieved using 30% methanol and 10% acetic acid solution –but impeach protein recovery-.
  The gel or pieces can be destained overnight if required for applications such as other detections, or extraction of proteins.

- **(optional)** The stained gel can be dried using Crack Free Solution #U50450 (for permanent records, autoradiography,...)

Comments for optimizations, and other applications

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

Gel %	Time (with shaking)
Protein gel <14%	3 x 10 minutes in 300mL water
Protein gel 14%-16%	3 x 30 minutes in 300mL water
Protein gel >16%	3 x 60 minutes in 300mL water

- **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.

- 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 agitation in a 50% methanol and 7% acetic acid solution for 15 minutes and then wash with ultrapure water.
- CooBlue (non fixed gels) is normally efficiently removed from proteins 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. Try staining after fixation of the gel (if not done)
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 (only10-15min) uses a microwave to speed the staining, while preserving sensitivity (10ng BSA). It is optimized for 1.0mm mini-gels. (For 1.5mm mini-gels, use the values in italic)

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

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

Related products and documents

* See all CooBlue products, including the

CooBlue FX-NoPreWash Protein Gel stain #1B8100: an analytical version (no fixation step, no pre-wash step, permanent staining)

CooBlue Blot-Membrane Protein Gel stain #20078A:

- Pump dispensor #T34711. This pump fits to the opening of the PP blue container of CooBlue 4.5L and delivers per push 10ml doses of reagent. It can be provided for free for your first order (please ask when ordering), or can be purchased separately.

* Protein Electrophoresis in Agarose Gels NT-47255g

- Crack free solution #U50450: to dry your gels without hassle of cracking.
- ProSave 5min Protein gel stain #BP7121: combines high sensitivity & max. flexibility (analytical, compatible with MS, elution, WB...)
- LavaPurple Gel & Blot protein stain #67433A: the highest sensitive protein gel stain (50ng protein) , by fluorescence (MS compatible).
- High quality reagents for electrophoresis (acrylamides, buffers...). See the catalog.

* Other Coomassie based reagents. I.e. CooAssay protein dosage kit: use the same Coomassie to quantitate proteins in solutions.

See BioSciences Innovations catalogue and e-search tool.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.

For any information, please ask: Uptima / Interchim Hotline: +33 4 70 03 73 06