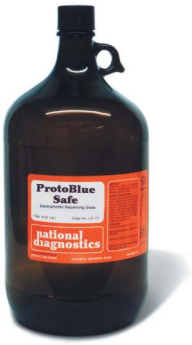
ProtoBlue™ Safe

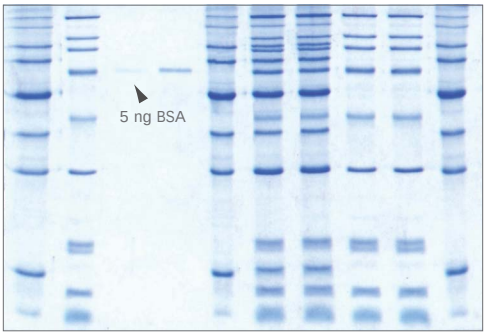
Eco-Friendly, Colloidal Coomassie® Stain

ProtoBlue Safe Colloidal Coomassie Blue G-250 stain is a premixed nonhazardous solution specially formulated for rapid, sensitive detection of proteins and safe, nonhazardous disposal. ProtoBlue Safe is the most sensitive Colloidal Coomassie stain on the market, with the ability to detect less than 5ng denatured BSA. ProtoBlue Safe contains no methanol, acetic acid, phosphoric acid or other hazardous components.



The Best Performing Colloidal Stain

The more finely controlled colloidal structure of ProtoBlue Safe improves both the sensitivity and the universality of staining compared to similar stains. ProtoBlue Safe is less prone to high background caused by trace residual SDS in the gel.



Samples separated on a 12% standard Laemmli gel, stained overnight with ProtoBlue Safe.

Lower Cost

Yearly Savings with ProtoBlue Safe

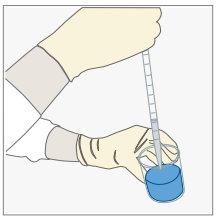
Calculations based on the list price purchase of the 4 liter ProtoBlue Safe versus the 3.5 liter size of the ecologically safe colloidal stain that is the current market leader (35ml of final stain solution per gel). Calculations include ethanol cost.

Nonhazardous Disposal

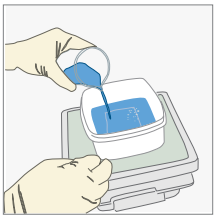
Used stain solution is not a hazardous waste (as defined by United States Title 40 Code of Federal Regulations (40 CFR 261.24(a)). Sink disposal of used stain solution is permitted in most locations.

Fast Protocol - No Methanol or HOAc

Wash gel 3 times for 5 minutes with deionized water on an orbital shaker.



Add 1 part ethanol to 9 parts staining solution while stirring. Standard denatured ethanol is fine.*



Add enough staining solution to completely cover the gel (20 - 50 ml).

Bands containing more than 1µg of protein will be detected within 15 minutes. For full sensitivity (up to 5ng), incubate the gel in stain for at least 5 hours.

** Adding the ethanol immediately before staining greatly improves the sensitivity, reproducibly and shelf life of ProtoBlue Safe as compared to formulations where the ethanol is added at the point of manufacture.*

Long Shelf Life - ProtoBlue Safe is stable for one year stored at room temperature in a cool, dry place.

ProtoBlue Safe	EC-722	1 Liter
		4 Liter

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Prepare Working Solution

Immediately before use, gently invert the bottle several times to resuspend colloidal dye particles that settle out on standing. To prepare working solution, add 1 part ethanol to 9 parts staining solution while stirring. A 20ml to 50ml volume of working solution is typically prepared, depending on the shape and size of the staining container.

Standard Protocol

Less than 5ng of denatured BSA can be detected by this protocol.

Stain mini gels in plastic containers reserved for this purpose that can hold about 100mls of solution. Gel fixation is not required. (If preferred, however, gels may be fixed in alcoholic or acidic solutions without affecting staining.)

1) Prepare ProtoBlue Safe Working Solution (above).
2) Wash the gel 3 times for 5 minutes each with deionized water on an orbital shaker.
3) After the last wash add enough ProtoBlue Safe stain to completely cover the gel.
4) Bands containing more than 1µg of protein will be detected within 15 minutes. For full sensitivity incubate the gel in stain for at least 5 hours. Longer incubations in the stain will not adversely affect the gel or staining sensitivity.
5) Remove the stain and wash the gel in deionized water. Incubating the gel in water increases sensitivity of detection by reducing the background to crystal-clear. The gel is stable in water for up to a week without loss of sensitivity. There is no need to store the gel in a salt solution.

Microwave Protocol

For fast staining - complete in 30 minutes. Twenty ng of denatured BSA can be detected after 10 minutes in water. Less than 5 ng can be detected after overnight incubation in water, due to a combination of bands binding up residual dye and the production of a crystal clear background.

All steps are performed in a loosely covered plastic container.

1) Prepare ProtoBlue Safe Working Solution (above).
2) Wash the gel in deionized water by microwaving for 45 seconds to one minute or until the solution starts to boil. Incubate for an additional minute on an orbital shaker.
3) Repeat the above step two more times. After the last wash rinse the gel in cold deionized water.
4) Add enough ProtoBlue Safe Working Solution to completely cover the gel. In a loosely covered container, microwave on high for 40 seconds in two approximately 20 second bursts. Stop if the solution starts to boil. Do not overheat the gel.
5) Shake the gel in the stain on an orbital shaker for 5-10 minutes for Laemmli gels (and MES or MOPS gels), and 10-20 minutes for ProtoGel Precision Precast Gels.
6) Remove the stain and rinse the gel several times. Incubate the gel in water on an orbital shaker until the required contrast/sensitivity is achieved. Optional: microwave gel in water for 1 minute to speed up contrast enhancement.

Frequently Asked Questions

Can the stain be re-used?

Re-use of the stain is not recommended.

Can membranes be stained?

Staining of membrane is not recommended because a high background is produced.

Can I use methanol instead of ethanol in the working solution?

Methanol can be used instead of ethanol provided it is added just before use and the standard protocol is used. With methanol, however, the working solution will no longer have the same hazard classification.

Is ProtoBlue Safe compatible with gel drying solutions?

Gels can be incubated for up to twenty minutes in drying solutions containing up to 20% ethanol with no detectable loss of staining sensitivity. Longer incubations will eventually lead to loss of staining sensitivity.