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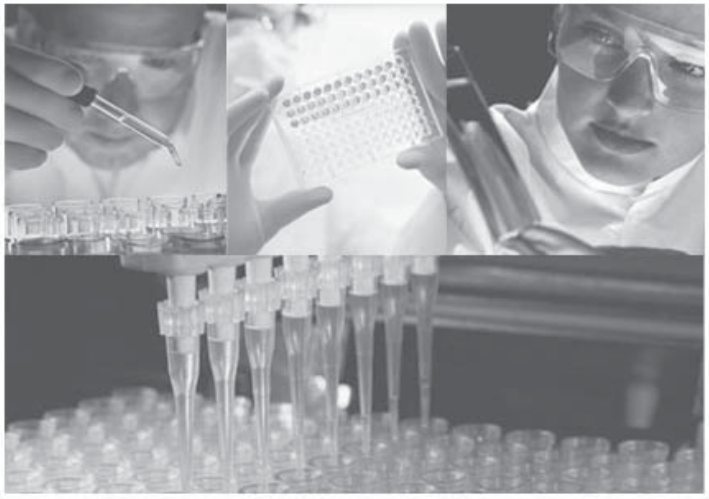

ProtoBlue™ Safe

Eco-Friendly Colloidal Coomassie Stain
Procedures for Gel Staining

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.

Procedures for gel staining using ProtoBlue Safe

ProtoBlue Safe stain formulation is compatible with all polyacrylamide gel types, and produces lower background staining than competing stains without requiring extensive water destaining to produce a crystal-clear background. It produces the type of intensely stained protein bands that previously required colloidal coomassie G-250 stains containing methanol.

Prepare Working Solution

1. Gently invert the bottle several times to resuspend colloidal dye particles that settle out on standing.
2. Add 1 part ethanol to 9 parts staining solution while stirring. (Standard denatured ethanol is fine). A 20ml to 50ml volume of working solution is typically prepared, depending on the shape and size of the staining container. To preserve solution, we recommend a plastic staining container just big enough to hold the gel.

Easy Protocol

This protocol requires the minimum hands-on time but may require longer staining times for maximum sensitivity.

1. Prepare ProtoBlue Safe Working Solution (above).
2. Rinse gel briefly in deionized water. Decant rinse water.
3. Add enough ProtoBlue Safe Working Solution to completely cover the gel.
4. Incubate for at least 6-8 hours. (The gel can be left overnight in staining solution if desired).
5. Wash the gel in water.

Standard Protocol

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

To conserve solution, choose a plastic container

1. Prepare ProtoBlue Safe Working Solution (above).
2. Wash the gel 3 times with 5 minutes each with deionized water on an orbital shaker. Decant wash solution.
3. After the last wash, add enough ProtoBlue Safe Working Solution 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 the stain for at least 4-5 hours. Longer incubations in the stain will not adversely affect the gel or the staining sensitivity.
5. Remove the stain and wash the gel in deionized water. Incubating the gel in water increases the 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. 20ng of denatured BSA can be detected after 10 minutes destaining in water. Less than 5ng can be detected after overnight incubation in water, due to a combination of bands binding residual dye and the production of a crystal clear background.

All steps are performed in a loosely covered plastic container. This protocol is optimized for 0.75mm thick Laemmli formulation mini gels.

1. Prepare ProtoBlue Safe Working Solution (above).
2. Wash the gel by microwaving in deionized water 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. Decant rinse 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 minutes. Gels thicker than 0.75mm may require longer incubations. 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.

Preparing Samples for Mass Spectroscopy

The following destaining solutions work with ProtoBlue Safe stained gel pieces being prepared for in-gel tryptic digests and mass spectroscopy.

- 25mM ammonium bicarbonate/50% acetonitrile
- 50mM ammonium bicarbonate/50% acetonitrile
- 100mM ammonium bicarbonate/50% acetonitrile

Procedure:

1. Cut out gel band or spot. Cut band into 1mm x 1mm pieces if necessary. Place in eppendorf tube.
2. Add 200µl destaining solution to gel pieces.
3. Incubate at room temperature or 37C for 30-45 minutes.
4. Remove destaining solution.
5. Repeat steps 1-3. Gel pieces should now be transparent.

Using 30% ethanol does not work satisfactorily.

ProtoBlue Safe Frequently Asked Questions

Is ProtoBlue Safe quantitative?
Yes. ProtoBlue Safe is quantitative. ProtoBlue safe gave a linear OD with protein concentration in a test covering three orders of magnitude.

Is ProtoBlue Safe compatible with mass spec?
ProtoBlue Safe is compatible with mass spectroscopy.

Can the stain be reused?
Reuse of the stain is not recommended. Components of the gel and running buffers reduce staining ability over time.

Some other bio-safe colloidal stains on the market already contain ethanol, but with ProtoBlue Safe the researcher needs to add the ethanol in forming the staining solution. Why did National Diagnostics choose to formulate ProtoBlue Safe this way?
Addition of ethanol at the time of use extends the shelf life of the stain and improves performance.

There are many types of SDS-PAGE gels, from traditional to long-life precast gels. Does ProtoBlue Safe work better with some gels than others?
No. ProtoBlue Safe performs comparably on all gel types tested. Thicker gels take longer to stain while lower percentage gels initially have higher background staining than high percentage ones.

Can ProtoBlue Safe be poured down the drain?
ProtoBlue Safe contains components that are not considered hazardous waste (as defined by United States Title 40 Code of Federal Regulations (40 CFR 261.24(a)) as well as EEC Directive 79/831/EEC Annex VII, JIS K 3363-1967). Observe state and local regulations.

Is the stain compatible with gel-drying solutions?
Gels can be incubated in gel drying solutions containing up to 20% ethanol for up to twenty minutes without loss of staining intensity. Longer incubations will eventually lead to destaining of bands.

Can I stain proteins transferred to membranes?
ProtoBlue Safe is not recommended for staining proteins on membranes because of the high background produced.

How does ProtoBlue Safe compare with Coomassie R-250 stains?
ProtoBlue Safe is faster, more sensitive, safer, and costs less. Furthermore, the ProtoBlue Safe stain solution may be disposed as nonhazardous waste in most locations.

I prepared more ProtoBlue Safe working solution than I need; can I save the unused portion for later use?
Unused working solution can be saved for later use by storing tightly capped in a bottle at room temperature.

Can I use ProtoBlue Safe on the gels that have already been stained by other methods?
Yes. ProtoBlue safe can be used to stain gels that have already been stained with Insite, Sypro stains or zinc stain.

Is the washing step before staining necessary?
The washing steps remove gel components that inhibit staining or may cause background staining. This step is not necessary but omitting the washing step will require longer incubation in the stain to achieve full staining.

I loaded 5ng of my protein in a lane but I cannot detect it with ProtoBlue Safe is something wrong?
The 5ng sensitivity reported is for 0.75mm gels containing denatured BSA. You may achieve higher or lower staining sensitivities depending on the protein being stained.

Can I stain isoelectric focusing gels with ProtoBlue Safe?
Yes. IEF gels can be stained with ProtoBlue Safe. Carrier ampholytes in the gel may cause high backgrounds. To avoid this the gels must be fixed in TCA before staining. Refer to the gel manufacturer instructions.

Can I store a gel stained in ProtoBlue Safe without drying it?
Gels stained in ProtoBlue safe can be stored in water for several days without losing sensitivity.

Can I use methanol instead of ethanol in the stain?
Yes but the stain solution would no longer be classified as non-hazardous for disposal.

Can I leave my gel in the stain over the weekend?
Yes. You can leave the gel in the stain over the weekend with only a slight increase in the background.

There is a precipitate at the bottom of the bottle is this normal?
ProtoBlue Safe contains colloidal particles that will normally settle out on standing. Gently invert the bottle several times to resuspend particles before use.

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