

# ProtoStain Blue

**national  
diagnostics**

## Colloidal Coomassie Stain

- Fast Protocol
- Nonhazardous; no methanol or acetic acid

ProtoStain Blue Colloidal Coomassie Blue G-250 stain is a premixed nonhazardous solution specially formulated for sensitive detection of proteins and safe, nonhazardous disposal. ProtoStain Blue is the most sensitive Colloidal Coomassie stain on the market, with the ability to detect down to 1 ng denatured BSA. ProtoStain Blue contains no methanol, acetic acid or phosphoric acid.

### Procedures for Gel Staining Using ProtoStain Blue

The ProtoStain Blue 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 clear background.

#### Standard Protocol (4-5 Hours)

As little as 1 ng of denatured BSA can be detected by this protocol. **Note:** *This protocol is optimized for a 0.75mm thick gel. Thicker gels require longer water washes.*

To conserve solution, use a plastic container.

1. Wash gel 3 times at 10 minutes each with deionized water on an orbital shaker. Decant wash solution. Longer washes give better consistency.
2. After the last wash add enough ProtoStain Blue solution to completely cover the gel. Invert bottle before use to resuspend particles before dispensing stain solution.
3. Bands containing more than 1  $\mu\text{g}$  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.
4. 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.

#### Rapid Protocol (60 Minutes)

For fast staining—complete in 60 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 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. Wash the gel by heating to 95°C in deionized water for 45 seconds to one minute. Incubate for an additional minute on an orbital shaker.
2. Repeat the above step two more times. After the last wash rinse the gel in cold deionized water. Decant rinse water.
3. Warm enough ProtoStain Blue solution to 65°C to completely cover the gel. Add warmed stain to the gel.
4. Shake the gel in the stain on an orbital shaker for 10-50 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

Gels stained with ProtoStain Blue can be destained for in-gel tryptic digests with 25-100mM 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 200  $\mu\text{l}$  destaining solution to gel pieces.
3. Incubate at room temperature or 37°C for 30-45 minutes.
4. Remove destaining solution.
5. Repeat steps 2-3. Gel pieces should now be transparent.

## ProtoStain Blue Frequently Asked Questions

### Is ProtoStain Blue quantitative?

Yes. ProtoStain Blue is quantitative. ProtoStain Blue gave a linear OD with protein concentration in a test covering three orders of magnitude.

### Is ProtoStain Blue compatible with mass spec?

ProtoStain Blue is compatible with mass spectroscopy.

### Can the stain be reused?

Reusing the stain is not recommended. Components of the gel and running buffers reduce staining ability over time.

### There are many types of SDS-PAGE gels, from traditional to long-life precast gels. Does ProtoStain Blue work better with some gels than others?

No. ProtoStain Blue 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 ProtoStain Blue be poured down the drain?

ProtoStain Blue 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 all 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?

ProtoStain Blue is not recommended for staining proteins on membranes because of the high background produced.

### How does ProtoStain Blue compare with Coomassie R-250 stains?

ProtoStain Blue is faster, more sensitive, safer, and costs less. Furthermore, the ProtoStain Blue stain solution may be disposed as nonhazardous waste in most locations.

### Is the washing step before staining necessary?

The washing steps remove gel components that inhibit staining or may cause background staining.

### I loaded 5ng of my protein in a lane but I cannot detect it with ProtoStain Blue. Is something wrong?

The 1 ng sensitivity reported is for 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 ProtoStain Blue?

Yes. IEF gels can be stained with ProtoStain Blue. 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 ProtoStain Blue without drying it?

Gels stained in ProtoStain Blue can be stored in water for several days without losing sensitivity.

### 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?

ProtoStain Blue 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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| <b>ProtoStain Blue</b><br>1 L | EC-727 |
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### For more information or order placement:

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|----------------------|------------------------------|
| <b>United States</b> |                              |
| Phone:               | (800) 526-3867               |
| Fax:                 | (404) 699-2077               |
| Email:               | info@nationaldiagnostics.com |
| <b>Europe</b>        |                              |
| Phone:               | 0115 982 1111                |
| Fax:                 | 0115 982 5275                |
| Email:               | sinfo@scientific-labs.com    |