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ab119211 InstantBlue® Coomassie Protein Stain

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: ISB1L

For the rapid Coomassie blue staining of protein in polyacrylamide gels.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

InstantBlue® is a ready-to-use, proprietary Coomassie® stain that is specially formulated for ultra-fast, sensitive and safe detection of your proteins. Protein gels can be stained in minutes without the need to wash, fix or destain.

Only proteins are stained resulting in well-defined blue bands on a highly transparent background. The reduction of background interference results in a better signal to noise ratio and may also have a positive impact on the overall resolution and sensitivity.

The InstantBlue® formulation is non-toxic and does not contain any methanol. Proteins stained using the InstantBlue® stain are also compatible with mass spectrometry (MS) analysis.

2. Storage and Stability

Upon receipt store at 4°C. Discard any reagents that show discoloration or evidence of microbial contamination. Be sure to keep the bottle capped when not in use. Once used, the staining solution should be discarded and cannot be reused.

3. Materials Supplied

Item	Quantity	Storage temperature
InstantBlue®® Protein Stain	1000 mL	4°C

Δ Note: *InstantBlue®® Protein Stain contains Coomassie dye, ethanol, phosphoric acid and solubilizing agents in water.* ***(Caution: Phosphoric acid is a corrosive liquid.)***

4. Technical Hints

InstantBlue® is provided as ready-to-use solution and should not be diluted.

Multiple washes prior to staining with InstantBlue® are NOT required or recommended.

An alcohol/acetic acid fixing step prior to staining with InstantBlue® is NOT required or recommended.

A destaining step post staining is NOT required or recommended with InstantBlue®.

5. Staining Protocol

Mix the InstantBlue® solution immediately before use by gently inverting the bottle a few times (do not shake the bottle to mix the solution).

5.1 Standard Protocol

1. After electrophoresis remove the gel from the tank and transfer directly into the InstantBlue® staining solution. Be sure that the gel moves freely in stain to facilitate diffusion. Typically, ~20 mL is needed to cover the gel.

2. Colored protein bands will start to develop immediately, and a suitable intensity is typically achieved after 15 minutes incubation at room temperature with gentle shaking.

3. Photograph your gel when the required intensity has been achieved. Gels can be kept in staining solution but ensure that the gel remains covered with liquid. Alternatively, the gel can be stored in ultrapure water after staining for 1 hour in InstantBlue®.

5.2 Protocol for gel drying

1. Ensure that the gel has been staining for at least 1 hour.

   Δ Note: *Although protein bands will be visible after a few minutes of incubation in stain, the staining process is typically fully completed after 1h incubation. Depending on the type of gel you are using longer incubation may be necessary. Further processing of the gel prior to completion of the staining process may result in protein destaining and reduced sensitivity. If this occurs simply restain the gel by incubating overnight in InstantBlue®.*

2. Submerse the gel in approximately 100 mL ultrapure water at ~70°C (heat for 30s to 60s in a microwave oven). Incubate for at least 1 hour while gently rocking. Optionally, adsorbent paper or paper towel can be added. Gels can be incubated overnight in ultrapure water.

3. Incubate the gel in a 'gel drying solution' (e.g. 4% glycerol, 20% ethanol in water) for 2 minutes. Incubation of any Coomassie®-stained gel in an alcohol solution will eventually result in destaining of the bands so avoid incubation for longer than 5 minutes.

4. The gel is now ready for drying between wetted cellophane membranes.

5.3 Protocol for destaining protein bands for MS analysis

1. Excise the protein band of interest and transfer to a clean sample tube.

2. Add 1 ml of 30% ethanol or 30% acetone or 30% acetic acid

   Δ Note: Acetic acid may result in acetylation of the N-terminus).

3. Incubate for 20 min (incubate at 60°C – 70°C to increase the rate of destaining).

4. Decant supernatant and repeat steps 5.3.2 & 5.3.3 at least 3 times or until gel is clear.

6. FAQs / Troubleshooting

1. What is the sensitivity of InstantBlue®?

It can detect as low as 5 ng of protein (BSA).

2. Does InstantBlue® have an upper limit of detection?

The stain does have an upper limit dependent on the volume of the InstantBlue® Stain used. The more InstantBlue®, the more Coomassie dye that is free to bind to protein. Once the Coomassie dye is exhausted, then it can't stain anymore protein. If that is ever the case, just simply add more stain and more dye will be bound. Approximately, 20-25 mL of stain can stain up to 40 µg of total protein per lane (12 lanes per gel).

3. Is it possible to restain the gel to reveal what's in the affected area? Also, would staining on a lab shaker eliminate the possibility of this happening?

Yes, they can restain with the same solution. A shaker would be great and if staining for a long time (like overnight) we say to put a lid on the staining dish so nothing dries out or oxidizes.

4. Does "fixing" of the protein occur during the incubation with InstantBlue®?

With InstantBlue® the fixing is occurring during the incubation with the stain. This is the reason why we recommend staining for 1 hour. Although bands are usually visible after 15 minutes they are not fixed at this stage. This is fine for people who just want to take an image or cut bands out for mass spec for example. But if they were to transfer the gel to water after this time then the stain would diffuse away from the protein. After around 1 hour the stain will be fixed in the gel.

5. Why isn't silver staining working following InstantBlue® coomassie stain and wash?

Probably need to wash gel 3x 100 mL water before silver stain. They are likely staining SDS sequestering agent. They could also leave gel in stain overnight to see fainter bands, but still need water wash before silver stain.

6. Is there any test to check if the InstantBlue® solution has been diluted?

Take a sample from a brand-new unopened bottle and a sample from the suspect bottle. Then add BSA to both samples to a final concentration of 1mg/ml. Both samples should now be blue. Dilute this and measure the absorbance at 600 nm in a UV spectrophotometer. You would need to experiment with the dilution ratio to get in the right range for the UV spec but it is going to be 10x or more. Both samples should be diluted the same amount. If UV absorbance of the suspect sample is much lower than the one from the new bottle, then yes, the suspected sample has been diluted.

7. InstantBlue® was left out on the lab bench over the weekend. Please let me know if this will affect product performance or shelf life?

Being at room temperature for the weekend will not affect the product performance or shelf life.

Technical Support

For all technical or commercial enquiries please go to:

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