InstantBlue™

For the Quickest, Sensitive and Safe Staining of Proteins

Introduction

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.

Contents

1L reagent, containing Coomassie dye, ethanol, phosphoric acid and solubilizing agents in water. (**Caution**: Phosphoric acid is a corrosive liquid.)

Storage

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.

Recommended Protocol and Notes on Usage

Before Use :

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

IMPORTANT

1) Multiple washes prior to staining with InstantBlue are **NOT** required or recommended.
2) An alcohol/acetic acid fixing step prior to staining with InstantBlue is **NOT** required or recommended.
3) A destaining step post staining is **NOT** required or recommended with InstantBlue.

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) Coloured 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. Close container to reduce evaporation of InstantBlue. Alternatively the gel can be stored in ultrapure water after staining for 1 hour in InstantBlue.

4) Once used, the staining solution should be discarded and cannot be reused. InstantBlue is provided as ready-to-use solution and should not be diluted.

Protocol for Gel Drying :

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

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 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.

Protocol for Destaining Protein Bands for MS analysis :

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

2) Add 1 ml of 30% ethanol or 30% acetone or 30% acetic acid
(Note: Acetic acid may result in acetylation of the N-terminus)

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

4) Decant supernatant and repeat step 2&3 at least 3 times or until gel is clear

For more detailed protocols please contact your MS facility.

Contact

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