

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) Submerge 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

Expedeon Protein Solutions
Unit 1A, Button End,
Harston,
Cambridgeshire CB22 7GX

Tel: +44 (0)1223 873364
Fax: +44 (0)1223 873371
scientist@expedeon.com

PLEASE READ THIS DOCUMENT CAREFULLY AS IT AFFECTS YOUR USE OF THIS EXPEDEON PRODUCT. IF YOU ARE UNWILLING TO ACCEPT THE TERMS SET OUT IN THIS DOCUMENT, PLEASE RETURN THE PRODUCT IN AN UNDAMAGED STATE ACCOMPANIED BY PROOF OF PURCHASE OR DELIVERY TO EXPEDEON LIMITED FOR A FULL REFUND OF THE PURCHASE PRICE PAID

By breaking the seal of this product's packaging, the Purchaser of this Kit (as defined below) agrees to the following terms:

Intellectual Property Licence

Certain reagents contained in the kit accompanied by this document (the "Kit") together with the method of use of the Kit are protected by intellectual property rights, including patents worldwide, ("Expedeon IP") which are owned by or exclusively licensed in certain fields to Expedeon Limited ("Expedeon").

The Purchaser is granted a non-exclusive, non sub-licensable licence (the "Licence") under the Expedeon IP, which is limited in the following ways:

The Purchaser may use the Kit solely for its internal research relating to protein analysis and/or preventing protein aggregation and may not re-sell the Kit or any reagents whether in whole or in part;

The Purchaser may not use the Kit in connection with the provision of services to third parties;

The Licence is limited to use of the Kit and expires upon exhaustion of the reagents contained in the Kit;

The Purchaser may not sub-license transfer or assign this Licence to any third party; and

This Licence shall terminate immediately if the product is used outside the scope of this Licence.

No other licence under the Expedeon IP is hereby granted whether expressly or by implication. All enquiries regarding the availability of licences for all other purposes should be addressed to Expedeon Limited, Unit 1A, Button End, Harston, Cambridgeshire CB22 7GX, United Kingdom. References to the Purchaser in this document shall also include any and all employees, officers, consultants and agents of the Purchaser who shall be bound by the terms of this document.

Intended Use

The Kit is solely for use in *in vitro* research and is not intended, and should not be used, in connection with any human or animal application, whether diagnostic, therapeutic, prophylactic or otherwise (including without limitation, for the production of any material which may be so used).

Disclaimer of Warranties

The Purchaser acknowledges that the Kit is experimental in nature and consequently Expedeon hereby excludes all representations and warranties whether express or implied with respect to the Kit, the reagents and information contained in the Kit, and use to be made of the Kit and in particular, but without prejudice to the generality of the foregoing Expedeon does NOT represent or warrant:

that using the Kit in accordance with the Licence will not infringe the intellectual property rights of any third party;

that using the Kit will result in any particular outcome or that any particular results will be achieved; or

that the Kit is fit for any particular purpose other than the purpose(s) specified in Expedeon 's instructions for this product

and the Purchaser acknowledges that no intended use for the product was made known to Expedeon.

In the event that the Kit does not conform to the specifications set out in the accompanying instructions or any other specification published by Expedeon (excluding representations made in marketing materials or oral representations made by Expedeon or its authorised representatives), the Purchaser's sole remedy as against Expedeon shall, at Expedeon 's election, be limited to the replacement of the Kit or a refund of the purchase price paid for the Kit.

Limitation of Liability

Expedeon accepts no liability to any party, whether in contract, tort, negligence, for breach of statutory duty or otherwise, for any loss of profits, business, contracts, anticipated savings, goodwill, or for any indirect or consequential loss or damage whatsoever, arising out of, or in connection with, this Licence or the subject matter of this Licence or use of the Kit.

The Purchaser is responsible for using the Kit correctly and should use it in accordance with Expedeon 's instructions for this product. Expedeon accepts no liability for damages or losses resulting from incorrect handling of the product or its components.

Nothing in this Licence shall exclude or limit Expedeon 's liability for death or personal injury arising as a result of Expedeon 's negligence, in respect of damage to property, death or personal injury arising from any fault or defect in the materials or workmanship of the Kit, or fraud. In all other cases, Expedeon 's liability in respect of each single incident giving rise to a claim against Expedeon, its employees or agents, shall be limited to the purchase price of the Kit.

Governing Law and Jurisdiction

This Licence shall be governed by English law and the Purchaser and Expedeon submit to the exclusive jurisdiction of the courts of England and Wales.