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A Geno Technology, Inc. (USA) brand name

Blue-*OUT*™

For the removal of Coomassie and fluorescent stain prior to mass spectrometry

(Cat. #786-683)

INTRODUCTION

Blue-*OUT*™ is a unique product that washes the interfering Coomassie and fluorescent protein stains from gel pieces prior to their treatment for protein sequencing or mass spectrometry.

ITEMS SUPPLIED

Description	Size
Blue-*OUT*	10ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature; store Blue-*OUT*™ at room temperature.

ITEMS NEEDED AND NOT SUPPLIED WITH THIS KIT:

- Ultrapure water (18MΩ equivalent), we recommend our Proteomic Grade Water (Cat. # 786-229)
- Mass Spectrometry Grade Acetonitrile
- Vacuum centrifuge (Speed-Vac®)
- 0.5ml clean microfuge tubes, we recommend treating with Protein-OUT™ (Cat. # 786-680), a unique solution to remove proteins and other mass spectrometry interfering agents

IMPORTANT INFORMATION

To reduce keratin and chemical backgrounds we recommend you wear gloves at all times and rinse them occasionally to reduce static build-up that attracts dust, hair and other interfering particles. Perform the entire process in a laminar flow hood, using tubes, tips and pipettes that were stored in the hood in a dust free environment. Avoid the use of detergents such as Triton and Tween (polymeric detergents) for cleaning flasks and glass plates used in electrophoresis.

Reduction & Alkylation will minimize artifactual peaks caused by disulfide bridges and side chain modifications and improve detection of peptides with cysteines. Alkylation by iodoacetamide will increase the mass of peptides by 57.02/cysteine present.

PROTOCOL

A. Excise Protein Spots/Bands

Processing of protein bands/spots. Following electrophoresis the proteins need to be fixed in the gel matrix. If a fixing step is not included with your staining technique we recommend fixing in 5% acetic acid in 1:1 ultrapure water: methanol. We recommend *LabSafe* GEL Blue™ (Cat. # 786-35), a mass spectrometry compatible Coomassie stain.

1. Rinse the entire gel in ultrapure water for 1-2 hours before processing.
2. Excise protein spots or bands with a clean scalpel and cut bands to 1-2mm cubes.
   NOTE: Pieces smaller than 1mm^2 may clog pipette tips in further processing.
3. Transfer to 0.5ml clean centrifuge tubes and briefly spin down in a benchtop centrifuge.
   NOTE: Tubes can be cleaned with with Protein-OUT™ (Cat. # 786-680), a unique solution to remove proteins and other mass spectrometry interfering agents.

B. Destaining of Proteins

NOTE: The Coomassie Destain protocol can also be used for fluorescent stained gels.

1. Add 100µl Blue-OUT™. Vortex and incubate for ~30 minutes, depending on the stain intensity. Vortex every 5-10 minutes during the incubation. Remove and discard the Blue-OUT™ solution.
   NOTE: The bulk of the Coomassie stain will be removed, however it is not necessary to complete destain the pieces.
2. Add 500µl acetonitrile and incubate at room temperature for 10 minutes, or until the gel pieces become opaque and shrink. Briefly centrifuge to pellet the gel pieces and remove all the liquid.
   NOTE: If required the gel piece can be treated with reducing and alkylating agents at this point. If not required, simply continue with the protocol. If required, a recommended protocol is shown in Appendix 1.
3. Add 500µl acetonitrile and vortex and incubate for 5 minutes. Discard the acetonitrile.
4. Repeat the acetonitrile wash (step 6) until the gel pieces are opaque white and completely dehydrated. This normally requires 2-3 washes.
5. Dry the gel pieces in a vacuum centrifuge and store at -20°C until use. The gel pieces can now be used for the in gel digestion of proteins and subsequent recovery of peptides.

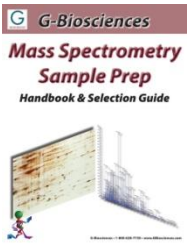
APPENDIX 1: DENATURING AND ALKYLATION

Preparation Before Use

1. Prepare a 10mM solution of DTT, we recommend our OneQuant™ DTT (Cat. # 786-077), single use aliquots:
   OneQuant™ DTT: Pierce an individual vial with a pipette tip and add 90µl Trypsin Digestion Buffer, to generate a 500mM solution. Vortex until completely dissolved. Dilute 1:50 with Trypsin Digestion Buffer to give a working 10mM DTT concentration. Store extra buffer at -20°C.
2. Prepare a 50mM iodoacetamide solution, we recommend our OneQuant™ Iodoacetamide (Cat. # 786-078):
   OneQuant™ Iodoacetamide: Pierce an individual vial with a pipette tip and add 150µl Trypsin Digestion Buffer to generate 500mM solution. Vortex until completely dissolved. Dilute 1:10 with Trypsin Digestion Buffer to give a working 50mM Iodoacetamide concentration. Make fresh each time.
3. Add 20-50µl DTT solution, ensuring the gel pieces are completely covered. Incubate at 60°C for 30 minutes.
4. Allow the tubes to cool to room temperature and then add 500µl acetonitrile and vortex and incubate for 5 minutes. Discard the acetonitrile.
5. Add 20-50µl iodoacetamide solution, ensuring the gel pieces are completely covered. Incubate at room temperature for 20 minutes in the dark.
6. Return to step 4 of the main protocol.

RELATED PRODUCTS

Download our Mass Spectrometry Handbook.



http://info2.gbiosciences.com/complete-mass-spectrometry-sample-preparation-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

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