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A Geno Technology, Inc. (USA) brand name

InGel™ Silver

For the In-Gel Tryptic Digestion of Silver Stained Proteins

(Cat. # 786-241)

INTRODUCTION

The InGel™ Silver kit provides a complete set of reagents for the in gel tryptic digestion and extraction of peptides for mass spectrometry (MALDI and LC MS/MS). The kit is specifically designed for use with silver stained protein spots/bands.

The first section of the protocol covers the in-gel digestion of proteins by our chemically modified Mass Spectrometry Grade Trypsin. Trypsin is a serine endopeptidase that specifically cleaves peptide bonds on the carboxy side of s-aminoethyl cysteine, arginine and lysine residues. Typically there is little or no cleavage at arginyl-proline and lysyl-proline bonds.

Trypsin naturally undergoes autolysis, producing trypsin fragments that interfere with sequence analysis. Our Mass Spectrometry Grade Trypsin is a chemically modified trypsin that is enzymatically active and yet resistant to autolysis. It is methylated, TPCK treated, affinity purified and quality tested for mass spectrometry.

The second part of the protocol focus on the extraction of the digested proteins from the gel pieces for analysis by mass spectrometry (MALDI and LC MS/MS). The InGel™ Silver kit is suitable for ~100 protein spots or bands.

ITEM(S) SUPPLIED (Cat. # 786-241)

Description	For 100 Spots/Bands
SilverOUT™ I	4ml
SilverOUT™ II	4ml
Trypsin Digestion Buffer	50ml
Pep-Extract™	4ml
Trypsin, Mass Spectrometry Grade	5 x 20µg
Trypsin Suspension Buffer	0.5ml
OneQuant DTT	5 vials
OneQuant Iodoacetamide	5 vials

STORAGE CONDITION

Shipped on blue ice. Store Mass Spectrometry Grade Trypsin at -20°C and store other components at 4°C.

ADDITIONAL ITEMS REQUIRED

- 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.
- 10% Trifluoroacetic acid

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.

Mass Spectrometry Grade Trypsin

This is chemically modified to prevent autolysis and therefore should not interfere with your mass spectral analysis. Under standard conditions, the most common trypsin fragment is 842.51 (m/z, M + H), which can be used as an internal standard.

Reduction & Alkylation

This 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 silver staining technique we recommend fixing in 5% acetic acid in 1:1 ultrapure water: methanol. For silver staining avoid the use of cross-linking reagents (i.e. glutaraldehyde) or strong oxidizers (i.e. chromates or permanganates). We recommend FOCUS™ FASTsilver™ (Cat. # 786-240), a mass spectrometry compatible silver 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. In-Gel Reduction, Alkylation & Destaining of Proteins

Preparation Before Use

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 unused DTT at -20°C.

OneQuant™ Iodoacetamide: Make fresh each time. 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.

Silver Destain, Denaturation and Alkylation

1. Prepare fresh working SilverOUT™ reagents by mixing equal volumes SilverOUT™ I and II.
   NOTE: *For each protein band you will require ~50µl.*
2. Add 50µl working SilverOUT™, ensuring the gel pieces are completely covered and vortex for 10 seconds. Incubate for 5-10 minutes or until the silver stain disappears from the gel band.
3. Remove working SilverOUT™ reagent and add 0.5ml ultrapure water, vortex and incubate for 5 minutes. Repeat the wash with ultrapure water until gel is clear.
4. 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.
5. Add 20-50µl DTT solution, ensuring the gel pieces are completely covered. Incubate at 60°C for 30 minutes.
6. Allow the tubes to cool to room temperature and then repeat step 4.
7. Add 20-50µl iodoacetamide solution, ensuring the gel pieces are completely covered. Incubate at room temperature for 20 minutes in the dark.
8. Add 500µl acetonitrile and vortex and incubate for 5 minutes. Discard the acetonitrile.
9. Repeat the acetonitrile wash (step 8) until the gel pieces are opaque white and completely dehydrated. This normally requires 2-3 washes.
10. Dry the gel pieces in a vacuum centrifuge and store at -20°C until use.

C. In Gel Trypsin Digestion

Preparation Before Use

Trypsin Rehydration Solution: To 1.2ml ultrapure water, add 150µl Trypsin Digestion Buffer and 150µl acetonitrile and vortex.

Trypsin Working Solution: Add 1.5ml Trypsin Rehydration Solution to the 20µg vial of Mass Spectrometry Grade Trypsin. Incubate for 15-30 minutes on ice and then gently pipette up and down to fully rehydrate. Do not vortex as this will lead to a loss of activity. The Trypsin should be solubilized immediately before use and unused Trypsin discarded.

NOTE: *If smaller volumes of trypsin are required, resuspend in an appropriate volume of Trypsin Suspension Buffer, incubate for 15-30 minutes on ice and then gently pipette up and down to fully rehydrate. Aliquot into 10µl aliquots and store at -20°C. To use, add 150µl Trypsin Rehydration Solution to each 10µl fraction.*

1. Add ~50µl Trypsin Working Solution to the gel pieces (enough to cover the gel pieces)
2. Incubate at 4°C for 30 minutes.
3. If all the Trypsin Working Solution has been absorbed then add more Trypsin Working Solution, ensuring the gel pieces remain covered.
4. Incubate for a further 90 minutes at 4°C and then add 10-20µl Trypsin Digestion Buffer to cover the gel pieces.
5. Incubate the digestion tube at 37^{o}C for overnight for maximal peptide recovery.

D. Peptide Extraction

For MALDI peptide mass mapping

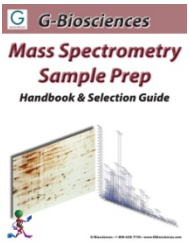
1. Cool the tubes to room temperature, centrifuge briefly in a benchtop centrifuge and remove a 1.5µl aliquot for MALDI peptide mapping.

For LC MS/MS

1. Briefly, centrifuge the digestion and add 40µl Pep-Extract™ and vortex. Incubate at 37°C for 15-30 minutes with periodic vortexing.
2. Centrifuge the tube briefly and collect the extract for analysis using a fine tip pipette to prevent removing the fine-gel particles that may clog analysis equipment. If samples require guanidation continue to the next section.
3. Dry the extra sample in a vacuum centrifuge and store at -20°C until use. Dried extracts can be stored for a few months.
4. To reconstitute for further LC MS/MS, add 10µl 0.1% trifluoroacetic acid and vortex. Remove the required aliquot for analysis and then dry the remaining sample in a vacuum centrifuge and store at -20°C.
5. The samples can now be guanidated.

RELATED PRODUCTS

Download our Mass Spectrometry Sample Preparation Handbook.



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

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