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A Geno Technology, Inc. (USA) brand name

FOCUS™ FASTsilver™

For Staining Protein Gels for Mass Spectrometry Analysis

(Cat. # 786-240,786-240T)

INTRODUCTION

G-Biosciences' FOCUS™ Fastsilver™ produces crystal clear backgrounds and maximal peptide recovery needed for critical analysis by mass spectrometry.

For mass spectrometry analysis, complete proteolytic digestion and recovery of peptides is required for optimal analysis, however silver ions in traditional silver staining kits inhibit proteolytic digestion. In addition, glutaraldehyde, a common sensitizer in silver stains, modifies peptide lysine residues preventing complete digestion and recovery. FOCUS™ FASTsilver™ produces high quality silver staining without the use of glutaraldehyde and is supplied with a highly efficient silver ion removal reagent, SilverOUT™. SilverOUT™ removes silver ions, which permits complete peptide digestion and extraction of peptides for maximal recovery.

ITEM(S) SUPPLIED

Description	Cat. # 786-240	Cat. # 786-240T
FOCUS™ Silver Stain	125ml	25ml
FOCUS™ Developer	75g	15g
Sensitizer-I	4ml	1ml
Sensitizer-II	4ml	1ml
SilverOUT™ Part-I	4ml	0.5ml
SilverOUT™ Part-II	4ml	0.5ml

STORAGE

The kit is shipped at ambient temperature. Store at room temperature upon arrival. Stable for 1 year when stored and handled properly. To ensure longer stability, FOCUS™ Developer has been supplied as dry powder.

ADDITIONAL REAGENTS REQUIRED

- Ethanol
- Glacial acetic acid
- Deionized water

IMPORTANT INFORMATION

- **POISON:** This kit contains heavy metal silver ions; avoid contact with skin and eyes. Wear gloves and eye protection.

- **NOTE**: Gel clarity will depend greatly on the quality and purity of the reagents used in making and running of the gel as well as the quality of protein sample loaded on the gel. Always use clean containers and highly purified de-ionized water for fixing and staining the gel. Never touch the gel with fingers.

PROTOCOL

1. After electrophoresis, fix the gel in a solution of 30% ethanol and 10% glacial acetic acid for 30 minutes to 3 hours. For isoelectric focusing gels, fix the gel first in 20% TCA for 30 minutes.
   NOTE: *The fixative incubation time is dependent on the thickness of the gel. For mini-gels, 20-25 minutes is sufficient.*
2. Wash the gel twice in 10% ethanol for 5-10 minutes each.
3. Wash the gel three times in deionized water for 5-10 minutes each.
   NOTE: *During washing steps 2 and 3, use generous amounts of the wash and use gentle rocking or agitation.*

Silver Staining

1. Add 5ml FOCUS™ Silver Stain to 45ml deionized water.
2. Next add 65µl of Sensitizer-I.
3. Soak the gel in the diluted Silver Stain for 20-30 min with gentle rocking of the gel, depending upon the gel thickness.

Development

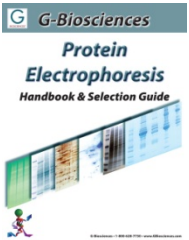
1. While the gel is staining, prepare the developer. Add two heaping spoonful (~3-4gm) of FOCUS™ Developer to 100ml of deionized water.
2. After the developer is dissolved, add 65µl of Sensitizer-I and 65µl of Sensitizer-II.
3. Rinse the gel 10-20 seconds with deionized water.
4. Add the Developer Solution to the gel and gently rock the gel until bands are visible. Band intensity will develop quickly.
5. As soon as band intensity reach an acceptable level, stop development with 2% acetic acid. Transfer the gel into the acetic acid solution and incubate for 10 minutes. Gel may be stored in 2% acetic acid or water.

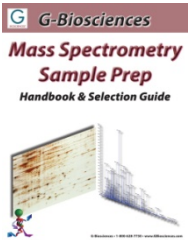
Destaining Silver Stain

1. Cut and remove the stained gel bands and transfer into clean microfuge tubes. Wash the gel bands with deionized water 3-4 times, 5 minutes each.
2. Prepare working SilverOUT™ reagents by mixing equal volumes of SilverOUT Part-I and Part-II. Make fresh reagent for each use.
   NOTE: *For each protein band, 50-75µl working SilverOUT™ is required.*
3. Add 50-75µl working *Silver*OUT™ on top of gel band and vortex for 10 seconds. Incubate for 5-10 minutes or until the silver stains disappear from the gel band.
4. Remove working *Silver*OUT™ reagent from the tube and discard. Add 1ml deionized water, vortex and incubate 5-10 minutes. Wash gel band with deionized water until gel band is clear. The gel band is ready for further analysis.

RELATED PRODUCTS

Download our Protein Electrophoresis or Mass Spectrometry Sample Preparation Handbooks.





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