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A Geno Technology, Inc. (USA) brand name

InGel Array™

(Cat. #786-241A)

INTRODUCTION

InGel Array™, Cat #786-241A**:** Consists of the following individual reagent components

- *Silver*OUT™ - De-staining reagents for protein gels stained with silver ions
- Trypsin Digestion Buffer - Trypsin Digestion Buffer for efficient digestion of protein with trypsin
- *Pep-Extract*™ - Buffer for extraction of peptides after digestion of protein with trypsin

A single instruction sheet describes the use of individual components of *InGel*Array™ kit - supplied with *Silver*OUT™, Trypsin Digestion Buffer, and *Pep-Extract*™. The kit contains sufficient reagents for extraction of ~500 protein spots/bands. For those who prefer to customize their own protocol, each reagent is also offered separately. Mass Spec grade modified Trypsin is offered separately (see related products).

The reagents do not contain metal salts ions or agents not suitable for MassSpec without further processing. However, protein digestion and peptide extraction are complex processes requiring many steps as well as clean supplies and laboratory procedures beyond our control, therefore, in some instances extracted peptide may require cleaning with C18 columns.

ITEMS SUPPLIED (Cat. # 786-241A)

Description	Size
*Silver*OUT™ Part-I	14ml
*Silver*OUT™ Part-II	14ml
Trypsin Digestion Buffer	2 x 14ml
Pep-Extract™	2 x 10ml
*InGel*Array™ Titer Plates (96 well)	5 Plates
*InGel*Array™ Strip Caps (12 strips/bag)	5 Bags

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store the kit components at room temperature.

ITEMS NEEDED BUT NOT SUPPLIED

- Pure water
- Siliconized tubes
- Centrifuge,
- Razor blades
- Acetonitrile
- Trypsin
- Vacuum pump

INSTRUCTIONS FOR USE

De-staining silver stain from protein gel spots (bands)

1. Transfer protein/gel spots (gel bands) into individual wells of the titer plate. Wash the gel spots with proteomic grade pure water twice, 5minutes each. [See Related Products for proteomic grade pure water]

2. Prepare working *Silver*OUT™ reagents by mixing equal volumes of Part-I, Part-II, and proteomic grade pure water. Make fresh reagent for each use.
   NOTE*- for each gel spot you would require 50µl of working Silver*OUT™*.*

3. Add 50µl of working *Silver*OUT™ on top of gel spot. Incubate for 5 minutes or until the silver stain disappears from the gel spots.

4. Remove *Silver*OUT™ reagent from the wells and discard. Add 0.1ml proteomic grade pure water and incubate for 5 minutes. Wash gel spots with proteomic grade pure water 4-5 times or until the gel spots are clear.
   NOTE*- for gels stained with methods other than silver ions; use an appropriate method to de-stain the gel spots. For Coomassie blue stained gels, wash the gel spots 4-5 times with 50% methanol in proteomic grade pure water.*

Dehydration Step

1. Following the de-staining, soak the gel pieces in 25µl Trypsin Digestion Buffer for 10 min. If necessary add 25-30µl proteomic grade water to submerge the gel pieces. Remove and discard the Trypsin Digestion Buffer.

2. Add 100µl MassSpec grade acetonitrile. Incubate for 5 minutes

3. Remove and discard acetonitrile. Repeat steps 2-3 twice or until the gel spots are opaque white and completely dehydrated.

4. Remove and discard acetonitrile. Dry the gel spots (air dry or vacuum assisted). Store the gel spots at –20°C until use.

Digestion of the protein-gel spots- Trypsin Digestion Buffer

NOTE*: - For digestion of protein-gel spots, it is recommended that the gel spots are de-stained, washed, and de-hydrated as described above.*

1. Prepare Trypsin -Trypsin Digestion Buffer: Transfer sufficient volume of Trypsin Digestion Buffer into a 0.5-0.6ml siliconized tube.

2. Prepare 50 µg trypsin /ml Digestion-Mix solution (use only MassSpec Grade trypsin). Use only freshly prepared trypsin.
   Note- the amount of trypsin used in any digestion may be modified at users preference.

3. Add 10-15µl Trypsin-Trypsin Digestion Buffer /well on the dry gel pieces. Incubate on ice for 50-60 minutes for the gel pieces to re-hydrate.

4. Remove and discard any remaining Trypsin-Trypsin Digestion Buffer with the re-hydrated gel pieces. Add Trypsin Digestion Buffer (5µl/well) only sufficient to keep the gel pieces wet during digestion. Close and seal the wells with the strip caps.

5. Incubate the digestion titer plate at 37^{o}C for 16-20 hours (overnight).

Extraction of peptides with Pep-Extract™

CAUTION- Open and use only one Pep-Extract™ vial at a time. This will reduce the risk of contamination and prolong the shelf life of the reagent. Close the vial immediately after use**.**

1. Following protein digestion, add 10-15µl *Pep-Extract™*.
   *(**NOTE**-condensation in the strip caps may be discarded by shaking the strip caps, this will minimize the extract volume)*

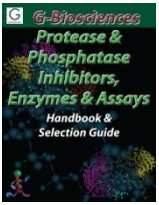
2. Freeze and thaw the titer plate twice at –20^{o}C. Transfer and incubate the titer plate at room temperature for 15 minutes.

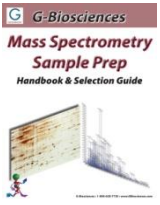
3. Use 1 µl peptide extract for MALDI analysis.
   NOTE- the extracts may be reduced in volume by vacuum drying.

4. The digestion extract may be removed and collected in separate vials. The gel spots may be extracted further 2-3 times with 5-10µl *Pep-Extract™* each. Pool the extracts in a tube. Vacuum dry to reduce volume to 5-10µl.
   NOTE: MALDI analysis will determine whether or not the extract is clean. If the extract requires cleaning, use C18 columns or HPLC separation.

RELATED PRODUCTS

Download our Protease Inhibitors and Mass Spectrometry Handbooks.





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