InGel™

[Silver*OUT™/ *Digestion Mix*™/ *Pep-Extract*™/ *MSG-Trypsin]

INTRODUCTION

InGel™ kit is supplied with ***Silver*OUT**™ – silver de-staining reagent, **Digestion Mix –** for efficient digestion of proteins with trypsin, ***Pep-Extract***™ – peptide extraction buffer and **MSG-Trypsin** (MassSpec Grade). The supplied reagents are sufficient for extraction of 100 protein bands. For those who prefer to customize their own protocol, each reagent is offered separately. This instruction sheet describes the use of individual components listed below.

These reagents do not contain metal ions and agents not suitable for MassSpec without further processing. However, protein digestion and peptide extraction is a complex process requiring many steps as well as clean supplies and laboratory procedure beyond our control, therefore, *G-Biosciences* does not guarantee clean preparation.

Kit COMPONENTS	**Cat # 786-241**	
***Silver*OUT**™ (Cat # 786-244)		
***Silver*OUT**™ **Part-I**	4ml	Store at RT
***Silver*OUT**™ **Part-II**	4ml	Store at RT
Digestion Mix (Cat # 786-242)	2 x 2 ml	Store at RT
Pep-Extract™ (Cat # 786-243)	4 x 4 ml	Store at RT
MSG (Mass Spec Grade) –Trypsin		
Procine (Cat # 786-245)	5 x 20µg	Store at -20°C
Trypsin Suspension Buffer	1 x 0.5ml	Store at -20°C

STORAGE CONDITION

All kit components are shipped at ambient temperature. Storage condition as mentioned above.

ITEMS NEEDED AND NOT SUPPLIED WITH THIS KIT:

Siliconized tubes, acetonitrile, de-ionized water, razor blades, centrifuge and vacuum pump

INSTRUCTIONS FOR USE

***Silver*OUT**™ (Cat # 786-244)

De-staining silver stain from protein gel bands

1. Cut and remove the gel bands for de-staining and transfer into clean microfuge tubes. Wash the gel bands with de-ionized water 3-4 times, 5 minutes each.
2. Prepare working ***Silver*OUT**™ reagents by mixing equal volumes of **Part-I** and **Part-II**. Make fresh reagent for each use. NOTE- for each protein band you would require 50-75µl.
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 stain disappears from the gel band.
4. Remove working ***Silver*OUT**™ reagent from the tube and discard. Add 1ml de-ionized water, vortex and incubate 5-10 minutes. Wash gel band with de-ionized water until gel is clear. The gel band is ready for further analysis.
5. We recommend the use of ***InGel***™ **-** for in-gel digestion of protein bands or MassSpec analysis (Cat # 786-241). See below for more information or visit our **web site** *www.GBiosciences.com* for more information.

OPTIONAL STEPS- Cut gel bands into small pieces & de-hydrate. Only needed if you plan in-gel protein digestion.

1. Following de-staining, soak gel pieces in (50-100µl) Digestion mix for 10 min. Remove and discard digestion mix. Cut gel bands into small pieces [1-2 mm sizes] with a clean razor blade.
2. Add 1ml MassSpec grade acetonitrile. Vortex and incubate for 5 minutes
3. Remove and discard acetonitrile. Repeat steps 2 to 3 twice or until gel pieces are opaque white and completely dehydrated.
4. Vacuum-dry the gel pieces and store at –20°C until use.

InGel™ **digestion of proteins in gel bands -Digestion Mix** (Cat# 786-242)

NOTE*: For In-gel digestion of protein bands, it is recommended that gel bands are de-stained, washed, cut into small pieces and de-hydrated as described above in the Optional Steps.*

1. Transfer the dried gel pieces/spots into 0.5-0.6ml siliconized tubes with cap.
2. Estimate volume (V) of the dry gel pieces in µl. Prepare three volumes Trypsin -Digestion mix (Vx3µl). Depending on gel pieces, an average ~ 30-60µl is sufficient.
3. **Preparation of Trypsin -Digestion mix**: Prepared 0.5µg/µl MSG-Trypsin solution with the supplied **Trypsin Suspension Buffer.**

   [IMPORTANT NOTE: *In some cases, after reconstitution, you would notice insoluble material, which is a* ***non-protein*** *and* ***will not interfere*** ***at all.*** *If you prefer to remove it, spin the tube briefly and take out the supernatant].*

   Add 1µl MSG-Trypsin (0.5µg/µl) solution in ~ 30-60µl **Digestion Mix** (in a siliconized tubes with cap). Mix the content by gently pipetting up/down and label the mix "**Trypsin-Digestion Mix**".

   Note*: The amount of trypsin used in any digestion may be modified at users preference (recommended ratio of enzyme to substrate is 1:100 to 1: 20).*
4. Add **Trypsin-Digestion Mix** on dry gel pieces and vortex. Incubate on ice for 50-60 minutes for gel pieces to re-hydrate.
5. Spin the digestion tube briefly for 10 seconds. Remove and discard any remaining Trypsin-Digestion mix with the re-hydrated gel pieces. Add 10-15µl Digestion mix (without Trypsin), only sufficient to keep the gel pieces wet during digestion. Close and seal the cap with parafilm.
6. Incubate the digestion tube at 37°C for 16-20 hours (overnight).

Extraction of peptides after *InGel*™ **digestion of proteins with *Pep-Extract***™ (Cat # 786-243)

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, briefly spin the digestion tube. Add 20-25µl *Pep-Extract*™, vortex and sonicate the tube for 3-5 minutes by placing the tube in a sonication bath. Incubate at room temperature for 15 minutes. Periodically vortex the tube.
2. Centrifuge the tube briefly and collect the extract for analysis - be careful and avoid fine-gel particles. Use 1µl for MALDI analysis.
3. The digestion sample may be extracted further 2-4 times with 25-30µl *Pep-Extract*™ each. Pool the extracts in a tube. Speed-vacuum to reduce volume to 5-10µl.

MALDI analysis will determine whether or not the extract is clean. If the extract requires cleaning use C18 columns or HPLC separation.

RELATED PRODUCTS

1. IEF/2D Electrophoresis Reagents & Buffers *- Save time and improve quality of IEF/2D electrophoresis with ready to use reagents and buffers. For details, please visit our web site www.GBiosciences.com or contact our tech dept.*

2. Agarose Sealing Solution (Cat # 786-226) *- For sealing IPG-strips while running second dimension gel, which can be used by simply heating and sealing the IPG strips for SDS-PAGE analysis.*

3. DTT (2M) (Cat # 786-227) and Iodoacetamide (Cat # 786-228) *- Ultra-pure proteomic grade 2M DTT in 5 x 1ml size, which can be simply added in buffer solution for maintaining protein reduction potential. An ultra-pure proteomic grade Iodoacetamide is provided in 5g sizes, which can be simply added in appropriate quantity in the reagent solution for alkylation of -SH group in proteins.*