

AGRO-BIO

EnzyBeads™ Trypsine

Version 2007/01

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Réactif/Reagent EnzyBeads™ Trypsine 100

Composition :

- 4 fl., EnzyBeads™ Trypsine 25 digestions, ready to use

Ref. MT03110

1- PRODUCT DESCRIPTION

EnzyBeads™ Trypsine offers a broad range of applications including protein identification and protein sequencing studies (**MSG-Chymotrypsine™** (Agro-Bio ref 786-13), **MSG-Lysine-C™** (Agro-Bio ref 786-14), etc...). Trypsin cleaves peptide bonds at the carboxylic side of lysine and arginine residues, creating peptides in the size range required for mass spectrometry analysis. The optimal pH of trypsin is between 7 and 9.

EnzyBeads™ Trypsine are able to digest large as well as very small quantities of proteins (e.g few nanograms). The lower limit of EnzyBeads™ Trypsine digestion is dependent upon the peptide detection method available in the laboratory.

2- COMPOSITION

- **EnzyBeads™ Trypsine** : 4 tubes containing a suspension of magnetic beads with immobilized Trypsin (Trypsin from bovine pancreas; TPCK treated; the treatment with L-1-Tosylamide-2-phenylethyl chloromethyl ketone (TPCK) inhibits the chymotrypsin activity).

3- PRECAUTIONS

- Preserved at 2-8°C under their origin state, the reagents are stable until the expiry date indicated on the kit or the reagent.
- The waste disposal will be carried out in accordance with the local regulation.

4- PREPARATION AND STABILITY OF THE REAGENTS

All the reagents must be kept at ambient temperature (18-25°C) during 30 minutes before their use. Use deionised water (conductivity 18.2 mΩ.cm⁻¹ at RT, 0.2µm filtered).

- **EnzyBeads™ Trypsine** : Reagent ready to use, to condition in Digestion Buffer before digestions (Cf. paragraph 6.2)
This reagent is stable for 1 month at 2-8°C after opening, when free of contaminations.

5- MATERIAL AND REAGENTS NECESSARY BUT NOT PROVIDED

| Ref. | Reagents and material not provided for certain kits | Reagents and material not provided |
|---------|--|---|
| MT03110 | <ul style="list-style-type: none"> EnzyBeads™ Magnet (Agro-Bio ref MT00110) | <ul style="list-style-type: none"> Digestion Buffer advised : Ammonium carbonate buffer (pH~8; ≈ 25mM). Prepare 15 mL of ammonium carbonate for 1 tube of EnzyBeads™ Trypsine 25 digestions. Centrifuge microtubes or all microtubes compatible with the magnet 5 µL to 200 µL adjustable pipettes Disposables tips for pipettes Deionised water (conductivity 18.2 mΩ.cm⁻¹ at RT, 0.2µm filtered). (Proteomic Grade Water - Agro-Bio ref 786-229). |

6- PROTOCOL**6.1 Preparation of the sample**

When identifying proteins, three parameters are important :

- The nature of protein solution to digest***

Enzymatic digestion of most proteins is facilitated after denaturation and alkylation of disulfide bonds. The protein reduction/alkylation (FOCUS™ Protein Reduction-Alkylation, Agro-Bio ref 786-231) allows their denaturation, the reduction of disulfide bonds and the alkylation of the free thiol functions.

- The technique used for peptide detection***

The peptide detection after digestion depends on the analysis device. For preferred peptide detection, the settings of the analysis device must be optimised.

- The protein solution concentration***

The protein solution concentration is important because it also conditions the detection of obtained peptides (see § 7, table 1: digestion results with EnzyBeads™ Trypsine). A too little concentrated protein solution will give few digestion peptides therefore hard to detect. The protein solution or the digestion peptide solution can be concentrated. (Tube-O-dialyzer™ and UPPA-PROTEIN-Concentrate™, Agro-Bio ref 786-142 and ref 786-120).

6.2 Digestion protocol

1. Conditioning of the reagent EnzyBeads™ Trypsine

- Homogenize the **EnzyBeads™ Trypsine** reagent by pipetting up and down.
- Transfer 25 µL of **EnzyBeads™ Trypsine** reagent directly to the bottom of a microtube of 0.5 mL avoiding the walls of the tube.
- Place the tube against the **EnzyBeads™ Magnet** for 20 seconds.
- Keep the tube on the **EnzyBeads™ Magnet** and remove the supernatant.
- Take the tube out of the magnet and resuspend the bead sediment in 100 µL of **EnzyBeads™ Digestion Buffer**.
- Homogenize well by pipetting up and down
- Place the tube against the **EnzyBeads™ Magnet** for 20 seconds.
- Keep the tube on the **EnzyBeads™ Magnet** and remove the supernatant.

Repeat this washing step twice and discard the supernatant.

2. Digestion

- Collect 5 µL to 100 µL of the protein solution diluted with **EnzyBeads™ Digestion Buffer**. The optimal volume for an optimal digestion is 50µL.
- Away from the magnetic field, pipette directly the protein solution onto the bead pellet in the microtube and thoroughly homogenize the magnetic beads in the protein solution by pipetting up and down.
- Incubate **at least** 15 minutes at room temperature (18-25°C), away from the magnetic field. Some proteins difficult to digest can require a longer digestion time. It is necessary in this case to carry out preliminary tests to determine the optimal digestion time.

3. Stopping the reaction

- Stop the enzymatic reaction by placing the tube on the **EnzyBeads™ Magnet**
- Keep the tube on the **EnzyBeads™ Magnet** and collect the supernatant containing the digestion peptides in a clean tube. Do not disturb the bead pellet. If beads are accidentally removed, release the whole solution and repeat step 3.

The digestion products can then be directly analyzed by mass spectrometry.

This digestion protocol is also compatible with any other method used in sample preparation.

7- RESULTS

• Digestions of pure proteins

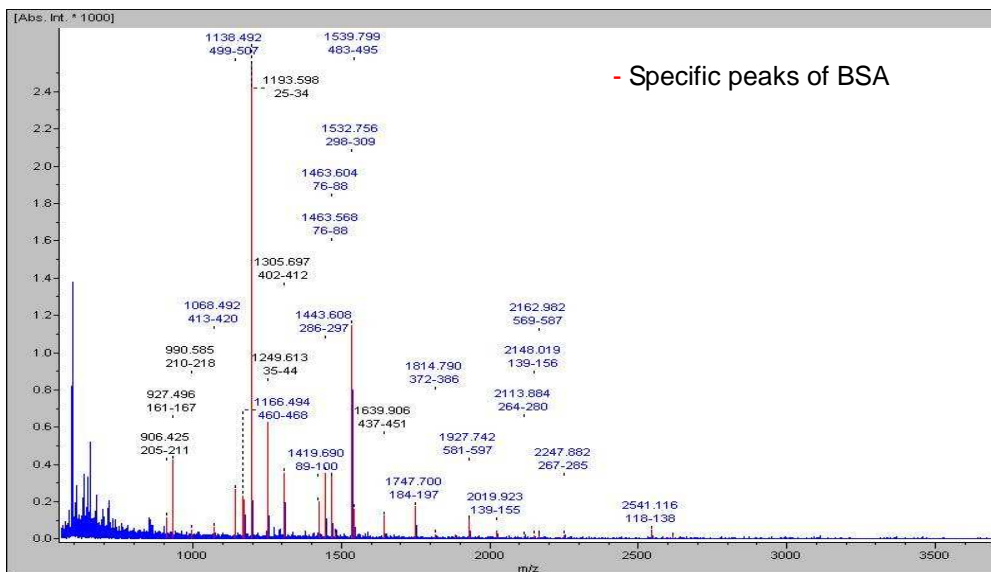
Digestions performed according to the **EnzyBeads™ Trypsine** protocol, 15 minutes at 18-25°C. Detection with a mass spectrometer Autoflex II TOF/TOF (Bruker-Daltonics GmbH, Bremen, Germany).

| Digested proteins | Concentration (mg/mL) | Detected quantity (pmol) | digested Volume (µL) | Peptide number | Score | Sequence coverage (%) |
|--------------------------------------|-----------------------|--------------------------|----------------------|----------------|-------|-----------------------|
| Transferrin 77 kDa | 0,2 | 0,95 | 5 | 20 | 106 | 21 % |
| Ovalbumin 42,8 kDa | 0,2 | 1,7 | | 9 | 62 | 23 % |
| Bovine Serum Albumin (BSA) 66 kDa | 0,01 | 0,05 | | 23 | 141 | 39 % |
| Carbonic anhydrase II 29 kDa | 0,4 | 4,9 | | 9 | 80 | 27% |

Tableau 1: Results of digestion by EnzyBeads™ Trypsine

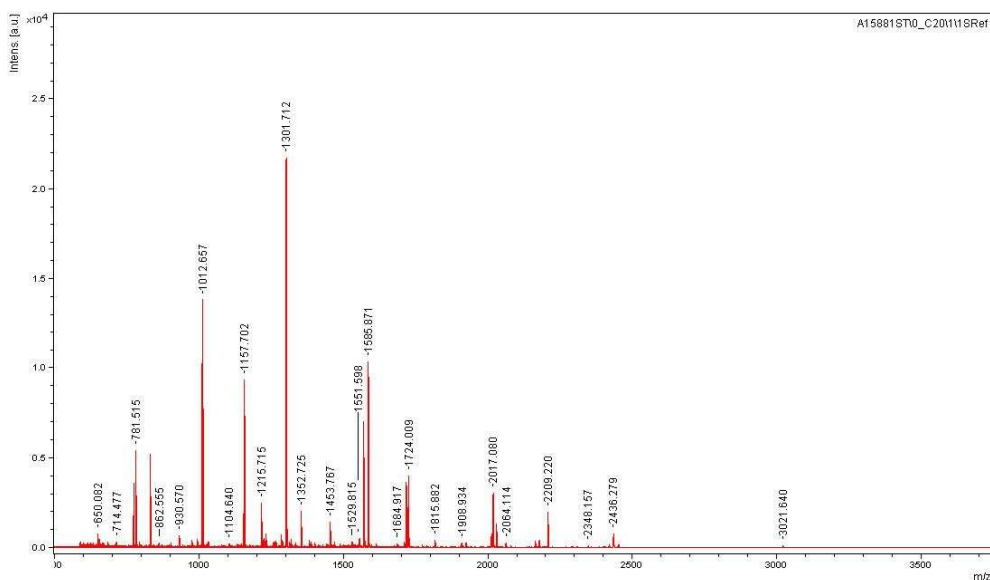
- MALDI mass spectrum of digest Bovine Serum Albumin (BSA) :**

Performed according to the **EnzyBeads™ Trypsine** protocol, 15 minutes at 18-25°C. Detection with a mass spectrometer Autoflex II TOF/TOF (Bruker-Daltonic GmbH, Bremen, Germany). The BSA sequence coverage is 42% with 29 detected peptides (cf spectrum 1: MALDI mass spectrum of a digested BSA).



- MALDI mass spectrum of digest complex sample**

A human serum (20 µL) was purified by hydrophobic chromatography on C8 beads then 5µL of the eluate was digested according to the **EnzyBeads™ Trypsine** protocol, 15 minutes at 18-25°C. Detection with a mass spectrometer Autoflex II TOF/TOF (Bruker-Daltonic GmbH, Bremen, Germany).



Spectre 2: MALDI mass spectrum of a digested serum

8- TECHNICAL SUPPORT

| Problems | Possible Causes | Suggestions |
|--|---|--|
| No or low detection of digested peptides | Low quantity of protein | Concentrate the sample to be digested (Tube-O-Dialyzer™ , Agro-Bio ref 786-142, UPPA-Protein Concentrate™ , Agro-Bio ref 786-120) |
| | | Increase the volume of EnzyBeads™ Trypsine and/or the digestion time. |
| | | Incubate the sample to digest and the EnzyBeads™ Trypsine at 37°C |
| | Concentrate the digestion supernatant (C18 Beads, C8 beads, ZipTip, ...). | |
| | Presence of trypsin inhibitors | Change the buffer containing the protein solution by dialysis (Tube-O-Dialyzer™ , Agro-Bio ref 786-142), diafiltration... |
| Bad matrix crystallization | Presence of salts | Use the Digestion Buffer preferably provided in the kit Adapt the volume of acidic water added to the digested sample in order to lower the salt concentration. |
| | Presence of beads in digested peptides | Use EnzyBeads™ Magnet (Agro-Bio ref MT00110) whose design is optimised not to take the beads when collecting digested proteins. |
| Contamination peaks | Presence of keratins or others contaminating proteins | Use only proteomic grade reagents. |
| | | Avoid siliconized tubes or any plastics that release polymers. |

- Temperature: the **EnzyBeads™ Trypsine** reagent can be used at 37°C to increase its performances on some proteins difficult to digest. Use the same protocol as at room temperature.
- Digestion Time: the minimum time is 15 minutes. For certain difficult proteins, it is necessary to carry out preliminary tests to determine the optimal digestion time.
- No agitation is necessary for digestion time lower than 30 minutes.
- No product of trypsin autodigestion appears to contaminate the analysis of the samples with **EnzyBeads™ Trypsine**. In order to calibrate your MALDI spectrum, use a statistical calibration, add your choice of calibration peptides or use the peaks of the matrix which appear on the spectra in normal conditions.
- It is recommended not to re-use the **EnzyBeads™ Trypsine** reagent in order to avoid any residual contamination due to previous digestions.

9- ASSOCIATED PRODUCTS

- **EnzyBeads™ Chymotrypsine** : Kit EnzyBeads™ Chymotrypsine 20 (Agro-Bio ref MT03200), Reactif/Reagent EnzyBeads™ Chymotrypsine 20 (Agro-Bio ref MT03205) : Digestion kits used for the digestion of proteins, for protein identification and protein sequencing studies.
- **NI protein Assay™** (Agro-Bio ref 786-005) : Protein Assays. An assay that overcomes interference of agents commonly present in protein solutions.
- **UPPA-Protein Concentrate™** (Agro-Bio ref 786-120) : For rapid precipitation and concentration of diluted protein solutions.
- **Tube-O-dialyzer™** (Agro-Bio ref 786-142) : no loss dialyzer for small and precious samples.
- **FOCUS™ Protein Reduction & Alkylation Reagents** (Agro-Bio ref 786-231) : Kits and reagents for disulfides reduction and thiols alkylation in protein samples.

INSTRUCTIONS

- **Complementary digestion enzymes** : MSG-Arginine-C™ (Agro-Bio ref 786-11), MSG-Aspartic-N™ (Agro-Bio ref 786-12), MSG-Chymotrypsin™ (Agro-Bio ref 786-13), MSG-Lysin-C™ (Agro-Bio ref 786-14), MSG-Glutamic-C™ (Agro-Bio ref 786-15). The use of various digestion enzymes allows protein sequencing and increases the sequence coverage leading to better protein identification.

10- BIBLIOGRAPHY

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11- LABELLING SYMBOLS

| | | | | | | | | |
|---|----|------------|---|----|--------|--|----|------------------------|
|  | GB | Batch code |  | GB | Use By |  | GB | Temperature limitation |
|---|----|------------|---|----|--------|--|----|------------------------|

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