

## PRODUCT INFORMATION

Trypsin NB Sequencing Grade, modified

Cat.No. 37283

### Product Description:

**General** Trypsin NB Sequencing Grade, modified is a serine endopeptidase which specifically cleaves at the carboxyl side of lysine, arginine and S-aminoethyl cysteine residues. There is little or no cleavage at arginyl-proline or lysyl-proline bonds. Cleavage may also be reduced when acidic residues are present on either side of a potentially susceptible bond [1].

**Application** Trypsin NB Sequencing Grade, modified is specially designed for digestion of proteins prior to mass spectrometric analysis.

**Features**

- Source: porcine pancreas
- Purity: > 90 %
- Tryptic activity: > 6000 U/g\*
- No chymotryptic activity detectable
- Modified by reductive methylation
- Quantity: ≥ 25 µg/vial, determined by measuring A<sub>280</sub>.

\*Unit definition: 1 U catalyzes the hydrolysis of 1 µmol Nα-Benzoyl-L-arginine-4-nitroanilide hydrochloride (BAPNA) per minute at 30 °C, pH 8.0.

**Stability** Trypsin NB Sequencing Grade, modified is more resistant towards autolysis even at pH values in weakly basic range (table 1). Therefore the enzyme can be used in high concentrations in the digestion assay.

| Incubation time (h) | Activity (%)   |                              |
|---------------------|--|------------------------------|
|                     | Trypsin NB Sequencing Grade, modified (Cat. No. 37283) | Trypsin native, not modified |
| 0                   | 100  | 100                          |
| 3                   | 100  | 43                           |
| 5                   | 87   | 30                           |
| 7                   | 84   | 25                           |
| 22                  | 46   | 5                            |

Tab.1: Stability of Trypsin NB Sequencing Grade, modified and Trypsin native, not modified in 20 mM Tris-HCl, pH 8.0 at 37 °C

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**TP Standard**     Trypsin Peptide (TP) Standard is delivered in a separate vial and contains trypsin to generate masses m/z 842 and 2211. If standard peptides are needed for internal calibration add to the trypsin digestion solution in a ratio: 1:5.

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**Storage conditions**     Trypsin NB Sequencing Grade, modified and TP Standard should be stored **in a dry state** at -15 to -25 °C.

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### Instructions for use:

**General**     Trypsin is routinely used in proteomics for mapping and protein sequencing due to its highly specific cleavage resulting in a limited number of tryptic peptides.

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**Digestion of proteins in solution**     Lyophilized Trypsin NB Sequencing Grade, modified is reconstituted in 25 µl 50 mM acetic acid to a final concentration of 1 µg/µl. For digestion of the target protein add Trypsin NB to a final protease:protein ratio of 1:100 to 1:20 (w/w).

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**In-gel protein digestion**     Lyophilized Trypsin NB Sequencing Grade, modified is reconstituted in 25 µl 50 mM acetic acid. Then add 475 µl 25 mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8 to a concentration of 50 µg/ml. For the final use dilute Trypsin NB solution 1:2.5 with 25 mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8 and use 10 to 20 µl for rehydration / digestion of each gel piece.

**Optional:** To avoid clogging of the LC system clear the solution from the In-gel digest by centrifugation of extract the peptides, e.g. with acetic acid and acetonitrile.

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