PRODUCT INFORMATION

Lysyl Endopeptidase® MS approved — Cat. No. 20987

Product description:

General

Lysyl Endopeptidase® (LysC) cleaves specifically the peptide bond on the C-terminal side of lysine (Lys) residues.

Application

LysC MS approved is for digestion of proteins prior to mass spectrometry analysis.

Features

- Appearance: Lyophilisate containing 2 mM Tris/HCl, pH 8.0
- Molecular weight: 27,000 (Gel filtration), 30,000 (SDS PAGE)
- Solubility: Soluble in water or buffer solutions
- Optimal pH: 9.0 - 9.5 (Amidase activity)
- Isoelectric point: 6.9 - 7.0
- Inhibitors: Diisopropylfluorophosphate (DFP), Phenylmethylsulfonyl fluoride (PMSF), Nα-Tosyl-L-lysine chloromethyl ketone hydrochloride (TLCK)

Storage conditions

LysC MS approved should be stored in a dry state at -15 °C to -25 °C (light protected).

Instructions for use:

Digestion of proteins in solution

Reconstitution of LysC:
Lyophilized LysC MS approved is reconstituted in 50 mM Tris/HCl pH 8.5 (final concentration of 1.0 µg/ml).
For digestion of the target protein add LysC to a final ratio of 1:100 to 1:20 (w/w) protease:protein

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In-gel protein digestion

Reconstitution of LysC

Lyophilized LysC MS approved is reconstituted in 50 mM Tris/HCl, pH 8.5 to a final concentration of 10 µg/ml.

Sample preparation:

- After electrophoresis, cut the protein band out of the gel and destain the gel pieces.
- Add 300 µl Acetonitrile (ACN) in a reaction tube and incubate the gel pieces 30 min while shaking on a mixer for dehydration.
- Remove the ACN and vacuum dry the sample for 15 min.
- Protein reduction:
  Add 100 µl 10 mM DTT in 100 mM NH_4HCO_3 and incubate 1 h at 56 °C.
- Let the sample cool down to room temperature and remove the DTT solution.
- Add 100 µl 50 mM Iodoacteamide in 100 mM NH_4HCO_3 and incubate 45 min in the dark with occasional vortexing.
- Wash the gel pieces 10 min with 100 µl 100 mM NH_4HCO_3.
- Add 300 µl ACN and incubate 15 min.
- Remove ACN, add 100 µl 100 mM NH_4HCO_3 and incubate 15 min.
- Remove solution, add 300 µl ACN and incubate 15 min.
- Remove ACN and vacuum dry the gel piece for 15 min.
- Add 100 µl of LysC solution (10 µg/ml) and incubate 45 min on ice.
- Remove LysC solution, add 10 µl 50 mM Tris/HCl, pH 8.5 and incubate the gel pieces overnight at 37 °C.
- Extract the peptides by shaking the gel pieces 20 min with 50 µl 20 mM NH_4HCO_3.
- Extract the peptides by shaking the gel pieces 3x 20 min with 5 % (v/v) formic acid in 50 % (v/v) ACN.
- If necessary, concentrate the peptides by vacuum drying, e.g. with SpeedVac.
- Desalt and purify the peptides with ZipTip®.
- If necessary, concentrate the peptides to 2 µl with weak vacuum.

Add the matrix and analyze it by mass spectrometry.

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