

Chymotrypsin sequencing grade

From bovine pancreas
salt free lyophilizate, EC 3.4.21.1

Cat. No. 11 418 467 001

4 × 25 µg

Version Jan. 2005

Store at +2 to +8° C

Product overview

Formulation	Lyophilized, salt-free.
Source	Chymotrypsin sequencing grade is isolated as a specific protease in ultra-pure from bovine pancreas
Specificity	Serine endopeptidase. At pH 7.0 – 9.0 it specifically hydrolyzes peptide bonds at the C-termini of Tyr, Phe, and Trp. Leu, Met Ala, Asp, and Glu are cleaved at a lower rate. The specificity of chymotrypsin sequencing grade is tested with melittin as substrate.
pH optimum	pH 7.0–9.0
Molecular weight	25 kDa
Purity	The enzyme is free of impurities which might interfere in the separation range of peptides in reversed-phase HPLC (detection at 215 nm). Function and purity control by HPLC and SDS polyacrylamide gel electrophoresis ensures a constant quality with each lot.
Contaminants	Trypsin < 0.07%
Inhibitors	Aprotinin, DFP, PMSF, phenothiazine-N-carbonyl chloride, TPCK, ZPCK, α ₂ -macroglobulin, α ₁ -antitrypsin, soybean trypsin inhibitor, and chymostatin. Note: No inhibition by APMSF.
Application	Hydrolysis of proteins by chymotrypsin alone or in combination with other proteases. Suitable for peptide mapping, fingerprinting, and sequencing analysis.
Storage/stability	Stable at +2 to +8°C, stored dry, until the expiration date printed on the label. A solution in 1 mM HCl can be stored for up to one week at +2 to +8°C.
Reconstitution	Dissolve lyophilized chymotrypsin sequencing grade in 1 mM HCl. The proteins to be sequenced are dissolved in digestion buffer (100 mM Tris-HCl*, 10 mM CaCl ₂ , pH 7.8).
Handling instructions	The content of one vial may be used for several simultaneous digests. In order to repeat the digest a new vial should be taken. Thereby the utmost reproducibility can be guaranteed and contamination will be avoided

Working concentration The recommended amount of enzyme is 1/200 to 1/20 of the quantity of protein by weight.

Incubation time The incubation time should be chosen between 2 and 18 h at 25°C depending on the amount of enzyme.

With the addition of guanidine hydrochloride or urea (final concentration up to 1 M, respectively) or acetonitrile [final concentration 5% (v/v)] the activity is maximally reduced by 20% (incubation time 5 h in the digestion buffer specified above).

The remaining activity after 1 h incubation in 0.01% SDS is 50 to 60%; in 0.1% SDS and 1% SDS respectively no more activity is found.

References

- 1 Blow, D. M. (1971) in *The Enzymes*, Vol. 3 (Boyer, P. D., eds.) pp. 185–212, Academic Press.
- 2 Kamp, R. M. (1986) in *Advanced Methods in Protein Microsequence Analysis* (Wittmann-Liebold, K. et al., eds.) pp. 8–20, Springer-Verlag.

* available from Roche Applied Science

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