Proteinase K - Solution

From Tritirachium album
Product No. A4392

Description

Molecular weight: 27000 g/mol
CAS-No.: [39450-01-6]
HS-No.: 35079090
EINECS: 2544578
E.C.: 3.4.21.14

Composition
Proteinase K: 20 mg/ml
CaCl₂ · 2H₂O 1 mM
Glycerol (v/v) 40 %
Tris · HCl (pH7.5) 10 mM

Specification
Activity: min. 600 mAnsonU/ml
DNases/RNases: not detectable

Unit definition: One milliAnson Unit (mAU) is the activity that releases folin-positive amino acids and peptides corresponding to 1 µmol tyrosine per minute.*

pH optimum: 7.5 - 12.0
pI: 8.9
Inactivation (temperature): > 65°C
Working concentration: varies from approx. 10 - 100 µg/ml
Stability (in solution): 12 months
Storage: 2-8°C; it is recommended to prepare aliquots for single use

* Alternative Unit definition: 1 DMC-unit catalyzes the hydrolysis of 1 µmol dimethyl casein per minute at 25 °C, pH 7.0. The liberated amino groups are determined with 2,4,6-trinitrobenzene sulfonic acid.

Comment

Proteinase K belongs to the family of the subtilisin type serine proteases. It shows endo- and exoproteolytic activity. Activated by calcium (1-5 mM), the enzyme cleaves proteins preferably behind hydrophobic amino acids (aliphatic, aromatic and other hydrophobic amino acids). Proteins will be completely digested, if the incubation time is long enough and the protease concentration is high. Temperatures above 65°C or the serine proteinase inhibitors AEBSF, PMSF or DFP inhibit the activity. Proteinase K is not inactivated by EDTA (see Ref. 5), iodoacetic acid or, interestingly, by other serine protease inhibitors like TLCK and TPCK.

Proteinase K is used to destruct proteins in cell lysates (tissue, cultured cells) and to liberate nucleic acids, since it very effectively digests DNases and RNases.
Application and Literature


(5) Proteinase K has two binding sites for Ca²⁺, located close to the active site, but not directly involved in the catalytic mechanism. Removal of the Ca²⁺ reduces the catalytic activity of proteinase K by 80 %. The residual activity is usually sufficient to degrade proteins that commonly contaminate preparations of nucleic acids. Therefore, digestions with proteinase K for nucleic acid purification are usually carried out in the presence of EDTA. If the presence of Ca²⁺ is necessary, add Ca²⁺ to 1 mM and remove later by addition of EGTA (pH 8.0, final conc. 2 mM). (Sambrock, J., Fritsch, E.F. & Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edition page B16. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.)