PanReac AppliChem
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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
$CaCl_2 \cdot 2H_2O$	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.

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Application and Literature

(1) Preparation of genomic DNA from bacteria (miniprep): bacteria from a saturated liquid culture are lysed and proteins removed by digestion with 100 µg/ml proteinase K for 1 hr at 37°C (Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl, K. (eds.) (1995) *Current Protocols in Molecular Biology.* Supplement 40 Page 2.4.1; Greene Publishing & Wiley-Interscience, New York).

(2) Whole-Mount in situ hybridization and detection of RNAs in vertebrate embryos and isolated organs: digestion of the sample e. g. in 10 µg/ml proteinase K for 15 min. at room tempera-ture; the length of treatment and/or concentration of the enzyme needs to be optimized! (Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl, K. (eds.) (1995) *Current Protocols in Molecular Biology.* Supplement 35 Page 14.9.3; Greene Publishing & Wiley-Interscience, New York).

(3) Preparation of DNA from cells or tissue samples for PCR: cells or tissue samples are incubated over night at 50°C with 100 µg/ml proteinase K (Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl, K. (eds.) (1995) *Current Protocols in Molecular Biology.* Supplement 17 Page 15.3.1; Greene Publishing & Wiley-Interscience, New York).

(4) Isolation of vaccinia virus DNA: digestion of the virus in a suspension with 2 mg/ml proteinase K for 4 hr at 37°C. (Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl, K. (eds.) (1995) *Current Protocols in Molecular Biology.* Supplement 43 Page 16.17.8; Greene Publishing & Wiley-Interscience, New York).

(5) Proteinase K has two binding sites for Ca^{2+}, located close to the active site, but not directly involved in the catalytic mechanism. Removal of the Ca^{2+} 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 Ca^{2+} is necessary, add Ca^{2+} 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.)

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