

Specification

Proteinase K

A3830

delivery form	lyophilized
origin	from <i>Tritirachium album</i>
Solubility (20°C)	soluble (H ₂ O)
M	27000 g/mol
CAS-No.:	39450-01-6
HS-No.:	35079090
EC-No.:	254-457-8
Storage:	2-8°C
LGK:	10 - 13
R:	36/37/38-42
S:	22-24-26-36/37
	irritant, sensitizing
WGK:	1
Specification	
DNases/RNases	not detectable
Activity/mg	min. 30 mAnsonU
Appearance	white
pH (1 %; H₂O; 20°C)	6.2 - 6.8
Literature	
<p>(1) Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl, K. (eds.) (1995) <i>Current Protocols in Molecular Biology</i>. Greene Publishing & Wiley-Interscience, New York</p> <p>(2) Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) <i>Molecular Cloning: A Laboratory Manual</i>, 2nd Edition page B16. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.</p> <p>(3) Müller, A. <i>et al.</i> (1994) <i>J. Biol. Chem.</i> 269, 23108-23111 Crystall structure of calcium-free proteinase K with a resolution of 1.5 Å.</p> <p>(4) Wallace, D.M. (1987) <i>Methods Enzymol.</i> 152, 41-48 Small and large scale phenol extractions.</p>	

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Comment

Proteinase K belongs to the family of subtilisin-like serine proteases. It has an endo- and exoproteolytic activity. Activated by calcium (1 - 5 mM), the enzyme digests proteins preferentially after hydrophobic amino acids (aliphatic, aromatic and other hydrophobic amino acids). Proteins will be completely digested, if the incubation time is long and the protease concentration high enough. Upon removal of the calcium ions, the stability of the enzyme is reduced, but the proteolytic activity remains (3). Proteinase K has two binding sites for Ca^{2+} , which are located close to the active center, but are not directly involved in the catalytic mechanism. Removal of the Ca^{2+} -ions reduces the catalytic activity of proteinase K by 80 %. The residual activity is sufficient to digest proteins, which usually contaminate nucleic acid preparations. Therefore, the digest with proteinase K for the purification of nucleic acids is performed in the presence of EDTA (inhibition of magnesium-dependent enzymes). Is the presence of Ca^{2+} required, Ca^{2+} is added up to a concentration of 1 mM and is removed by the addition of EGTA (pH 8.0; final conc. 2 mM) later on. The pH-optimum is at 8, but the enzyme is active over a wide pH-range (pH 4.3 - 12). An elevation of the reaction temperature from 37°C to 50 - 60°C may increase the activity several times, like the addition of 0.5 - 1 % SDS. Temperatures above 65°C, trichloroacetic acid or the serine protease-inhibitors AEBSF, PMSF or DFP inhibit the activity. Proteinase K will not be inhibited by EDTA (see ref. 2), urea (1 - 4 M), SDS, citrate, iodoacetic acid or, interestingly, by other serine protease inhibitors like TLCK and TPCK. In case that proteinase K has to be inactivated, make sure, that the temperature is not below 95°C and the time not shorter than 10 minutes. A TCA-precipitation is well suited too.

Proteinase K is used for the destruction of proteins in cell lysates (tissue, cell culture cells) and for the release of nucleic acids, since it very effectively inactivates DNases and RNases. Some examples for applications: Purification of genomic DNA from bacteria (miniprep): Bacteria from a saturated liquid culture are lysed and proteins are removed by a digest with 100 µg/ml proteinase K for 1 h at 37°C (ref. 1 Suppl. 40 page 2.4.1); Whole-Mount *in situ* hybridization and determination of RNAs in vertebrate embryos and isolated organs: Digest of the sample with e. g. 10 µg/ml proteinase K for 15 minutes at room temperature; The period of the treatment and/or the concentration of the enzyme has to be optimized (ref. 1 Suppl. 35 page 14.9.3); Preparation of DNA from cells or tissue for PCR: Cells or tissue are incubated over night at 50°C with 100 µg/ml proteinase K (ref. 1 Suppl. 17 page 15.3.1); Isolation of vaccinia virus DNA: Digest the virus in a suspension with 2 mg/ml proteinase K for 4 h at 37°C (ref. 1 Suppl. 43 page 16.17.8); Before the phenol extraction for the purification of nucleic acids is performed, a digest with proteinase K may be introduced (50 - 200 µg/ml final concentration; 37°C for 30 minutes in the presence of SDS; ref. 4). We recommend a working concentration between 10 - 100 µg/ml.

Stability: Lyophilized proteinase K is stable at +4°C for at least 12 months. In solution, the stability is approx. 6 - 12 months at +4°C to -20°C. Stock solutions (10 - 20 mg/ml) may be prepared in 10 mM CaCl_2 or 50 mM Tris · HCl, pH 8.0; 1 mM CaCl_2 or 50 % Glycerol; 20 mM Tris · HCl, pH 7.4; 1 mM CaCl_2 or 50 % Glycerol; 50 mM Tris · HCl, pH 8.0; 1 mM CaCl_2 .