Worthington Biochemical Corporation

PROTEINASE K

Molecular Biology/Protease

PRODUCT HIGHLIGHT

Proteinase K is a serine endopeptidase with a broad spectrum of action originally isolated from the fungus *Tritirachium album limber.* Worthington Recombinant Proteinase K is supplied as a highly purified lyophilized powder (PROKR) and ready-to-use liquid (PROKRS), and tested to be free of DNase and RNase contaminants.

Description	Activity	Code	Catalog No.	Size
Proteinase K, Recombinant, Solution, 20mg/ml A concentrated, ready to use liquid formulation. Proteinase K prepared at 20mg/ml in 10mM Tris-HCl, 1mM calcium acetate, pH 7.5 containing 50% glycerol, DNase and RNase free. Store at -20°C.	≥ 400 units per milliliter ≥ 20 units per mg dry weight	**PROKRS**	LS004254 LS004256 LS004258	5 ml 25 ml Bulk
Proteinase K, Recombinant, Lyophilized A lyophilized powder. Purified to remove DNase and RNase. Store at 2-8°C.	≥ 20 units per mg dry weight	**PROKR**	LS004248 LS004249 LS004250 LS004252	25 mg 100 mg 1 gm Bulk

Characteristics of from Recombinant *Tritirachium album* Protease K produced in yeast

Molecular weight: 28,900 daltons.

pH Optimum: Stable over a wide pH range: 4.0-12.5, optimum pH 7.5-8.0, using denatured hemoglobin as substrate.

Stability: Although calcium ions do not affect the enzyme activity, they do protect Proteinase K against autolysis and increase thermal stability when present at a concentration of 1 - 5 µmoles. An interesting characteristic of Proteinase K is that it retains its activity in the presence of sodium dodecyl sulphate (SDS) or urea. (0.5 - 1% SDS and 1 - 4 M urea). Raising the temperature of the reaction from 37°C to 50 - 60°C can increase the activity several fold. A special feature of Proteinase K is its ability to digest native proteins, thereby inactivating enzymes such as DNase and RNase without recourse to a denaturation process. Proteinase K is inactivated by diisopropyl fluorophosphate (DFP) or phenyl methane sulphonyl fluoride (PMSF). Chelating agents such as citrate and EDTA have no affect on the enzyme activity. Proteinase K can also be inactivated by heating above 65°C for 15-20 minutes or by extraction with phenol/chloroform.

Storage: The lyophilized powder is stable for ≥1 year at 2-8°C. Solutions in 50mM Tris-HCl, pH 8.0 with 1mM CaCl2 are stable for months at 2-8°C.

Unit Definition: One Unit releases one micromole of Folin positive amino acids, measured as tyrosine, at 37°C, pH 7.5, using urea denatured hemoglobin as the substrate.

Specificity: In addition to cleavage of peptide bonds, it is able to catalyze peptide amide hydrolysis.

Application: The recommended working concentration for Proteinase K is 0.05-1 mg/ml.
Proteinase K is very useful in the isolation of highly native, undamaged DNAs or RNAs, since most microbial or mammalian DNases and RNases are rapidly inactivated by the enzyme, particularly in the presence of 0.2 - 1% SDS.

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Proteases For Protein Digestion and Protein Sequencing

Enzyme	Specificity	Molecular Weight KDa	pH Optimum	Extinction Coefficient E1%, 280nm	Common Substrates	Activators	Inhibitors	Product Code/ Applications
Proteases For Protein Sequencing								
Carboxy-peptidase B	H2-N-Rn-Y-†-X-COOH X = basic amino acids (Arg, Lys, Orn) Y = nonspecific	34.3	7.0-9.0	21.4 (Folk 1971)	Hippuryl-L-arginine	None required	EDTA Hg^{2+} & other heavy metal ions EDTA, EGTA *o*-phenanthroline	**COBC/COBPMS,** Sequence analysis by successive cleavage of C-terminal basic amino acids Insulin production
Carboxy-peptidase Y	H2-N-Rn-Y-†-X-COOH X ,Y= non-specific, prefers aromatic	64.0	4.5-6.0	15.0 (Hayashi *et al.* 1973, and Kuhn *et al.* 1973)	ATEE Bz-Phe-Ala-Leu Z-Phe-Ala	None required	APCK, Aprotinin DFP 4-Hydroxymer-curibenzoate PMSF	**COY,** C-terminal sequencing & Modification/ labeling of peptides and proteins
Chymotrypsin TLCK treated	-X-†-Y- X = aromatic Y = nonspecific	25.6	7.8-8.0	20.57 (Theoretical)	ATEE BTEE	None required	α-antitrypsin Aprotinin DFP, PMSF, TPCK α2-macroglobulin	**CDSEQ, CDTLCK,** Sequence analysis Peptide synthesis, mapping/finger-printing
Endo-Arg-C (Clostripain)	-Arg-†-Y- Y = nonspecific	53	7.4-7.8	16.57 (Theoretical)	BAEE	Ca^{2+} Reducing agents	EDTA, TLCK, Tris Hg^{2+} & other heavy metal ions	**CPSEQ, CP,** Peptide mapping & synthesis Sequence analysis Hydrolysis/ condensation of amide bonds
Endo-Glu-C (Staph. Protease V8)	-Glu-†-Y- (NH4 buffers pH 4, 7.8) -Asp-†-Y- (PO4 buffer pH 7.8)	27.0	4.0 & 7.8	4.26 (Houmard 1976)	Casein Z-Phe-Leu-Glu-4NA	None required	DFP F-, Cl-, Br-, CH3COO- NO3-α2-macroglobulin	**STSEQ, STAP,** Peptide mapping & sequence analysis
Endo-Lys-C	-Lys-+-Y- Y= nonspecific	30.0	7.0-9.0	18.63 (Theoretical)	N-p-Tosyl-Gly-Pro-Lys pNA	None required	DFP, TLCK, Aprotinin, Leupeptin	LYS-C, LYSEQ Peptide mapping and sequence analysis
Pepsin	-X-†-Y- X = nonspecific but aromatic & hydrophobic preferred Y ≠ Ala, Gly, Va	34.6	1.0-4.0 unstable ≥5	14.39 (Theoretical)	Casein Hemoglobin	None required	Pepstatin A Diazoketones Epoxides	**PM,** Collagen bioprocessing/ purification Antibody cleavage
Proteinase K	-X-†-Y- X = nonspecific but aliphatic, aromatic & hydrophobic preferred Y = nonspecific	28.9	7.5-12	12.6 (Theoretical)	Casein Hemoglobin Keratin	Ca^{2+} Active in 0.5-1% SDS	DFP EGTA PMSF	**PROKR, PROKRS,** DNA/RNA purification
Trypsin	-X-†-Y- X = Arg, Lys Y = nonspecific	23.8	7.5-8.5	14.3	BAEE Casein TAME	Ca^{2+} Lanthanide	Aprotinin, Benzamidine DFP, EDTA, Leupeptin α2-macroglobulin PMSF, TLCK Trypsin Inhibitors (egg white, lima bean, pancreatic, soybean)	**TRTPCK, TRSEQZ, TRSEQII,** Peptide mapping & sequence analysis Cleavage fusion proteins

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