Proteinase K

GE010.0100 – 100 mg

Proteinase K is a broad-spectrum serine protease (28.9kDa monomer) that cleaves peptide bonds at the carboxylic sides of aliphatic, aromatic, and hydrophobic amino acids.

Quantity

100 mg lyophilized powder purified from purified from *Pichia pastoris* harbouring the gene encoding endolytic protease from *Tritirachium album*.

Applications

Isolation of genomic DNA from cultured cells and tissues, removal of DNAses and RNAses during DNA and/or RNA purification, determination of enzyme locations

Specifications

Free of DNAses and RNAses. Specific Activity ~30 Kunitz units per mg.

Quality Control

Enzyme activity is assayed by digesting hemoglobin at a concentration of 16,7 mg/ml in a solution of 0.08M potassium phosphate (pH 7,5), 5M urea, 4mM NaCl, 3mM $CaCl_2$. Absence of endodeoxyribonucleases, exodeoxyribonucleases, and ribonucleases was confirmed by appropriate assays.

Storage

For short term, store lyophilized powder at +4ºC. Before use, reconstitute Proteinase K at a desired concentration using either ultrapure water or 50mM Tris-HCl pH 7,5 containing 5mM $CaCl_2$ and 50% glycerol. Reconstituted Proteinase K should be stored at -20ºC.

Enzyme activity

Proteinase K is activated by calcium (1-5mM). Although Ca^{2+} does not directly influences catalytic activity it does contributes to the protein stability (protection against autolysis, increasing thermal stability). Removal of Ca^{2+} (e.g. by adding EDTA) reduces proteolytic activity by 80%, however, 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 such as DNAses). Proteinase K is also stable over a wide pH range (4-12), with a pH optimum of pH 8.0. Elevation of the reaction temperature from 37°C to 50 - 60°C may increase the activity several times, as might the addition of 0.5 - 1% SDS, 3M of Guanidinium chloride, 1Mof Guanidinium thiocyanate, and 4M urea. The recommended working concentration of Proteinase K is 0,05-1 mg/ml.

Inhibition

Proteinase K can be inhibited by phenylmethylsulfonyl fluoride (PMSF), trichloroacetic acid (TCA), 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), and diisopropyl phosphorofluoridate (DFP or DIFP). Proteinase K **is NOT** inhibited by metal chelators, thiol-reactive reagents, or by specific trypsin or chymotrypsin inhibitors (including SDS, Tween-20, Triton X-100, urea, EDTA, citrate, iodoacetic acid, Sarkosyl, Guanidinium chloride, Guanidinium thiocyanate, TLCK, and TPCK)

Inactivation

Proteinase K can be heat-inactivated at temperatures above 65˚C

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