Proteinase K
(with storage buffer)

#GE010.0100 (100 mg) | GE010.1000 (1g)
(FOR RESEARCH ONLY)

Product: 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.

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.

Content: #GE010.0100 contains 100 mg lyophilized powder purified from *Pichia pastoris* harbouring the gene encoding endolytic protease from *Tritirachium album*, supplied with 1ml of a 10x concentrated storage buffer, whereas #GE010.1000 contains 1g lyophilized powder, supplies with 10ml of a 10x concentrated storage buffer.

Properties: Free of DNases and RNases.
Specific Activity: >30 units per mg.

QC: Enzyme activity is assayed by digesting hemoglobin at a concentration of 16.7 mg/ml in a solution of 80mM potassium phosphate (pH 7.5), 5M urea, 4mM NaCl, 3mM $CaCl_2$. Absence of endodeoxyribonucleases, exodeoxyribonucleases, and ribonucleases was confirmed by appropriate assays

Storage: Store lyophilized powder at +4ºC for several months or -20ºC for at least 2 years. The concentrated (10x) storage buffer (500mM Tris-HCl, 50mM $CaCl_2$) can be stored at room temperature or +4ºC.

Prior to use:

Reconstitute Proteinase K at a desired concentration (e.g. 10 mg/ml) using either ultrapure water or 1x Storage Buffer (50mM Tris-HCl pH8.0) [dilute storage buffer with ultrapure DNase/RNase-free water with or without glycerol (see below)]. Reconstituted Proteinase K should be stored at +4°C for up to a few months. If longer storage is required, reconstitute Proteinase K in 1x Storage Buffer containing 50% glycerol and store at -20°C

Usage:

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 50-56°C (Proteinase K is active in the temperature range of 20-60°C with optimum 50-56°C) may increase the activity several times, as might the addition of 0.5-1% SDS, 3M of Guanidinium chloride, 1M of 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