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ProductInformation

Proteinase K
from *Engyodontium album*
(formerly *Tritirachium album*)
For Molecular Biology

Catalog Number **P4850**
Storage Temperature 2–8 °C

CAS RN 39450-01-6
E.C. 3.4.21.64
Synonym: Endoproteinase K

Product Description

Proteinase K is a stable serine protease with broad substrate specificity. It degrades many proteins in the native state even in the presence of detergents. Proteinase K was isolated from a fungus able to grow on keratin and the enzyme can digest native keratin (hair), hence, the name "Proteinase K".[1] The predominant site of cleavage is the peptide bond adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked alpha amino groups. It is commonly used for its broad specificity.[2,3,4] The mode and specificity of action has been studied.[4]

Proteinase K is frequently used in molecular biology applications to digest unwanted proteins, such as nucleases from DNA or RNA preparations from microorganisms, cultured cells, and plants.[5-11] The enzyme is typically used at 50-200 µg/ml in nucleic acid preparations at pH 7.5-8.0 and 37 °C. Incubation times vary from 30 minutes to 18 hours. Proteinase K is usually denatured by subsequent phenol extractions, although it can autodigest during long incubations.[3] This product is tested for suitability in molecular biology applications.

Proteinase K is active in 1% TRITON® X-100 and fully active in 0.5% (w/v) SDS. SDS and urea will denature protein substrates resulting in increased digestion rates. Proteinase K itself is denatured much more slowly by these agents.[3,12,13]

Molecular mass: 28,930 Da (amino acid sequence)[14]
28,500 Da (SDS-PAGE)[15]

pH range: 7.5 to 12.0 (urea-denatured hemoglobin as substrate), but most often used in pH range 7.5-9.0.[2,3]

Temperature profile: maximum activity at 37 °C (activity is >80% of maximum between 20–60 °C)[3]

pI:[2] 8.9

Extinction coefficient: $E^{1\%}$ = 14.2 (280 nm, 10 mM NaCl and 5 mM $CaCl_2$, pH 8.0)[2]

Activators: 1-5 mM Ca^{2+} is required for activation. When calcium is removed from the enzyme (addition of EDTA) 25% of the catalytic activity is lost. However, if the EDTA-Ca^{2+} complex is removed from the enzyme solution by gel filtration, a total of 80% of the enzyme activity is lost and only a small activation will occur upon addition of excess Ca^{2+} to the Ca^{2+}-free enzyme.[16]

Inhibitors: Proteinase K is inhibited by DIFP or PMSF (the latter used at final concentration 5 mM).[3] It is partly inactivated, but not inhibited, by EDTA (see Activators). Proteinase K is not inhibited by iodoacetic acid, the trypsin-specific inhibitor TLCK, the chymotrypsin-specific inhibitor TPCK, and *p*-chloromercuribenzoate.

This product is supplied as a solution of 10 mM Tris-HCl, pH 7.5, with 40% glycerol and 1 mM calcium acetate.

Specific activity: >800 units/ml

Unit Definition: One unit will hydrolyze urea-denatured hemoglobin to produce color equivalent to 1.0 µmole (181 µg) of tyrosine per minute at pH 7.5 at 37 °C.

Enzymatic Impurities:
Endo-Exonuclease – none detected
Endonuclease (nickase) – none detected
RNase – none detected
DNA – <500 picograms DNA/mg solid (<0.5 ppm)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store the product at 2–8 °C. It is stable for at least 2 years.

References

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