Certificate of Analysis

PRODUCT:

Proteinase K (Recombinant) Solution
***Tritirachium* alkaline proteinase**
[Endopeptidase K]

PRODUCT NUMBER: P-1264-SOL

LOT NUMBER: L1492

DESCRIPTION:

Proteinase K is a highly active 28,904-Da serine protease isolated from the fungus *Tritirachium album*. The enzyme exhibits broad cleavage specificity on native and denatured proteins and is widely used in the purification of DNA and RNA. Its activity is increased in the presence of denaturants such as SDS (1%) and elevated temperature (50-60°C). The recommended working concentration is 50-100 µg/ml for protein removal and enzyme inactivation (efficient removal of proteins from nucleic acid solutions), and up to 2 mg/ml for tissue treatment. The Proteinase K, Lyophilized powder can be prepared as a 20 mg/ml stock solution in water and stored in aliquots at -20°C. This product is free of detectable DNase and RNase.

UNIT DEFINITION:

One unit is defined as the amount of enzyme that will hydrolyze urea-denatured hemoglobin to produce color equivalent to 1.0 µmol tyrosine per minute @ 37°C, pH 7.5 (color by Folin-Ciocalteu reagent).

SPECIFIC ACTIVITY: >30 units/mg.

PURITY: >99%

RNase ACTIVITY:

Contaminating RNases: RNase activity not detectable as determined by incubating RNA with Proteinase K for 6 hours at 37°C.

DNase ACTIVITY:

Contaminating DNases: Nicking activity not detectable as determined by incubating pBR322 with Proteinase K for 6 hours at 37°C.

EXONUCLEASES: Not detectable

APPLICATIONS:

- Facilitate the purification of nucleic acids by degradation of contaminating proteins in yeast, bacteria, tissue lysates and mammalian cell lysates.
- Inactivation of enzymes such as DNase and RNase in sample materials, to favor the isolation of high molecular weight nucleic acids.
- Topology probe for the location and orientation of membrane-bound proteins.
- Improving PCR cloning efficiencies.
- Inactivation of enzyme cocktails in ribonuclease protection assays.

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OPTIMAL CONDITIONS FOR USE:

Proteinase K requires calcium ions for stability but not for catalytic activity. Some published procedures specify the addition of $CaCl_2$ from a freshly prepared stock, to a final concentration of 10 mM just prior to proteolytic digestion. The enzyme is active in the pH range from 7.5–12 and in the presence of up to 1% SDS and up to 4 M urea. The enzyme retains activity at temperatures in excess of 60°C and peak catalytic activity is seen in the range from 50–60°C.

INHIBITORS:

Proteinase K is inactivated by serine protease inhibitors such as phenylmethylsulfonyl fluoride (PMSF) or diisopropylfluorophosphate (DFP) as well as the more water soluble, reduced toxicity serine protease inhibitors such as AEBSF (Prod.# A-1018). It is not inactivated by metal ion chelating agents such as EDTA, or EGTA. Proteinase K activity is also unaffected by sulfhydryl reagents PCMB, TLCK, or TPCK.

REACTION CONDITIONS:

Proteinase K will cleave native proteins but exhibits higher proteolytic activity when proteins are denatured with a chaotropic agent and treated with a disulfide-bond reducing agent such as β-mercaptoethanol or dithiothreitol. The protocol below is presented as a starting point but reactions conditions will vary widely depending on the type of starting material. For example, Proteinase K treatment of immobilized tissue sections may be incompatible with the chaotropes and heat treatment described. Consult published protocols for recommended reaction conditions for specific applications.

1. Denature up to 10 mg of protein by treating at 95°C in 1% SDS, or treating with 6 M guanidine-HCl or 8 M urea dissolved in a buffer containing 50 mM Tris-HCl, pH 8.0, 5 mM DTT.

2. If using urea or guanidine, dilute the sample until the chaotrope is 2 M or less.

3. Prepare a 15 mg/ml (100X) concentrated stock of Proteinase K in 50 mM Tris-HCl, pH 8.0 with 1 mM CaCl.

4. Add 0.01 volume of Proteinase K concentrate to the sample and incubate at 40–60°C for 1–2 h. Stop the reaction by treatment with PMSF or similar serine protease inhibitors. Alternatively, when working with soluble nucleic acid samples, Proteinase K can be removed by extraction with phenol:chloroform:isoamyl alcohol (25:24:1).

STORAGE & HANDLING:

Store at -20°C. ***WARNING:*** **HARMFUL! IRRITANT! AVOID FREEZING/THAWING CYCLES!**

CAUTION: For research use only. Not for human or drug use. The pharmacological and toxicological properties of this product have not been fully investigated. Use caution when handling. Do not use this compound if you are not fully trained or are unaware of the hazards involved.

Verified: *PC*

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