Certificate of Analysis

Proteinase K:

Part No.	Size
V302B	100mg

Part# 9PIV302

Revised 7/13

Description: Proteinase K, produced by the fungus *Tritirachium album* Limber, is a serine protease that exhibits a very broad cleavage specificity. It cleaves peptide bonds adjacent to the carboxylic group of aliphatic and aromatic amino acids and is useful for general digestion of protein in biological samples (1). It has been purified to be free of RNase and DNase activities. The stability of Proteinase K in urea and SDS and its ability to digest native proteins make it useful for a variety of applications, including preparation of chromosomal DNA for pulsed-field gel electrophoresis (2), protein fingerprinting (3,4) and removal of nucleases from preparations of DNA (5) and RNA (6,7). A typical working concentration for Proteinase K is 50–100µg/ml.

Molecular Weight: 28,900 daltons.

Reconstitution: Proteinase K is supplied as a lyophilized powder. Reconstitute in 50mM Tris-HCl (pH 8.0), 10mM $CaCl_2$.

Source: *Tritirachium album* Limber.

Specific Activity: ≥30mAnson units/mg protein. See Product Information Label.

Storage Conditions: See the Product Information Label for storage recommendations. The lyophilized powder should be stored desiccated. The reconstituted protease should be stored at –20°C, where it is stable for 2–3 months. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes. These fluctuations can greatly alter product stability. It is best to prepare proteases just prior to use or aliquot and freeze at –20°C. See the expiration date on the Product Information Label.

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Promega

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Quality Control Assays

Activity Assay: An amount of 2.2µg of Proteinase K is assayed in reaction buffer containing 33.3mM HEPES (pH 7.5), 1mM $CaCl_2$ and 4.8mM Suc-Gly-Gly-Phe-pNA in a final volume of 0.75ml. Kinetics are recorded for 3 minutes at a wavelength of 405nm.

Exonuclease Assay: To test for DNase or RNase activity, 50ng of radiolabeled DNA or RNA is incubated with 20µg of Proteinase K for 3 hours at 37°C in 0.77X MULTI-CORE™ Buffer. The release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is ≤3% release for both DNase and RNase.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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Printed in USA. Revised 7/13

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Usage Information

1. Reaction Conditions

Protein Denaturation: In general, proteins require denaturation and disulfide bond cleavage before enzymatic digestion can go to completion. Proteinase K displays strong proteolytic activity on denatured proteins and on native proteins as well (1).

1. Dissolve 1–10mg of the target protein in 6M guanidine-HCl (or 6–8M urea), 50mM Tris-HCl (pH 8), 2–5mM DTT (or β-mercaptoethanol) in a reaction volume of up to 1ml (25µl minimum).
2. Heat at 95°C for 15–20 minutes or at 60°C for 45–60 minutes. If smaller amounts of protein are to be digested, the recommended conditions given can be scaled down proportionally. However, under no conditions should less than 25µl of the dissolving agent be used.
3. After denaturation, allow the reaction to cool and add 50mM Tris-HCl (pH 7.5), 5mM $CaCl_2$ until the guanidine-HCl or urea concentration is below 2M.

Protease Digestion: Add Proteinase K to the reaction to a final concentration of 50–100µg/ml. Incubate at 37–56°C for at least 1 hour. Reducing the temperature to below 37°C will decrease the digestion rate. Longer incubations of up to 24 hours may be required, depending on the protein. If using longer incubations, one must be very careful to avoid bacterial contamination.

To terminate the reaction, add an inhibitor of Proteinase K such as PMSF (1) or DFP. The reaction can also be terminated by the addition of EGTA (pH 8.0) to a final concentration of 2mM or by TCA precipitation. Proteinase K may not be completely inactivated by EGTA, as this enzyme retains partial activity in the absence of calcium (7). Heat treatment (10–15 minutes at 65°C) only partially inactivates Proteinase K (inhibition by no more than 20–25%).

Protein Cleavage and Nuclease Removal: Proteinase K can be used to cleave native proteins and to remove nucleases from DNA (5) or RNA (6,7) preparations. If digestion of a nondenatured (native) protein is desired, incubate the protein with Proteinase K at a concentration of 50–100µg/ml at 37–56°C in 50mM Tris-HCl (pH 7.5), 5mM $CaCl_2$ or another buffer that is compatible with the stability of the target protein.

To remove nucleases from DNA/RNA preparations, incubate the nucleic acid with Proteinase K at a concentration of 50µg/ml at 37°C in 0.01M Tris (pH 7.8), 5mM EDTA, 0.5% SDS (7).

Inhibitors: Phenylmethylsulfonyl fluoride (5mM PMSF; 1,8), Diisopropyl phosphorofluoridate (DFP), EGTA. Proteinase K is not inhibited by EDTA, iodoacetic acid, TLCK or TPCK.

Stability: Proteinase K is a very stable protease, active in wide pH and temperature ranges. The protease is active in a pH range of 4.3–12.0, with optimal activity at pH 8.0. Proteinase K has a broad temperature profile, retaining >80% of its activity at temperatures of 20–60°C (8). The protease is active in SDS concentrations as high as 0.5%. Calcium is a stabilizer of Proteinase K; however, when Ca^{2+} is removed from the enzyme, 20% of the catalytic activity may still remain (7). This may be enough activity to degrade proteins commonly found in nucleic acid preparations. The enzyme is also active in 1% (w/v) Triton® X-100 (8).

2. References

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