rProteinase K Solution AOF

Instructions for Use of Product A5055.

Quick Protocol

Proteinase K is a serine protease that exhibits broad cleavage activity. It cleaves peptide bonds adjacent to the carboxylic group of aliphatic and aromatic amino acids and is useful for general protein digestion in biological samples. rProteinase K Solution AOF is cGMP-manufactured Animal Origin Free (AOF) from *Pichia pastoris* cells expressing a recombinant clone.

Reaction Conditions

Protein Denaturation

In general, proteins require denaturation and disulfide bond cleavage for complete enzymatic digestion. rProteinase K Solution AOF 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 25µl–1ml.
2. Heat at 95°C for 15–20 minutes or at 60°C for 45–60 minutes. If digesting smaller amounts of protein, 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 dilute the guanidine-HCl or urea concentration to less than 2M by adding 50mM Tris-HCl (pH 7.5), 5mM $CaCl_2$.

Protease Digestion

Add rProteinase K Solution AOF 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, carefully avoid bacterial contamination.

To terminate the reaction, add a Proteinase K inhibitor such as PMSF (1) or DFP. The reaction can also be terminated by adding 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 (20–25% inhibition).

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 digesting 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 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% activity at temperatures of 20–60°C (8). The protease is active in SDS concentrations as high as 0.5%. Calcium stabilizes Proteinase K; however, in the absence of Ca^{2+}, 20% of the catalytic activity can 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).

References

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