

## Product Information

### Proteinase K from *Tritirachium album*

Catalog Number **P5568**  
Storage Temperature 2–8 °C

CAS RN 39450-01-6

E.C. 3.4.21.64

Synonyms: Peptidase K, Endoproteinase K,  
Endopeptidase 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 its name "Proteinase K".<sup>1</sup> Evidence from crystal and molecular structure studies indicates the enzyme belongs to the subtilisin family with an active-site catalytic triad (Asp<sup>39</sup>-His<sup>69</sup>-Ser<sup>224</sup>). 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.<sup>2-4</sup> The mode and specificity of action has been studied.<sup>4</sup>

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.<sup>5-11</sup> 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 removed by subsequent phenol extractions, although it can autolyse during long incubations.<sup>3</sup>

Proteinase K has been used to remove endotoxins bound to cationic proteins such as lysozyme and ribonuclease A.<sup>12</sup> It has been used for determination of enzyme localization on membranes,<sup>13</sup> treatment of paraffin embedded tissue sections to expose antigen binding sites for antibody labeling,<sup>14</sup> and remove nucleases for *in situ* hybridization.<sup>15</sup> Research on prions in Transmissible Spongiform Encephalopathies (TSE) and proposed diagnostic tests utilize Proteinase K digestion of proteins from brain tissue samples.<sup>16,17</sup> Protease footprinting by Proteinase K digestion can reveal protein-protein surface interactions.<sup>18</sup>

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.<sup>3,19,20</sup>

Molecular mass: 28,930 Da (amino acid sequence)<sup>21</sup>  
28,500 Da (SDS-PAGE)<sup>22</sup>

pH range:<sup>2,3</sup> 7.5–12.0 (urea-denatured hemoglobin as substrate), but most often used in pH range 7.5–9.0.

Temperature profile:<sup>3</sup> maximum activity at 37 °C  
(In the temperature range of 20–60 °C, a minimum of 80% of the activity observed at 37 °C is expected)

pI:<sup>2</sup> 8.9

Extinction coefficient:<sup>2</sup>  $E^{1\%} = 14.2$   
(280 nm, 10 mM NaCl and 5 mM CaCl<sub>2</sub>, pH 8.0)

Activators: 1–5 mM Ca<sup>2+</sup> is required for activation. When calcium is removed from the enzyme (by addition of EDTA), 25% of the catalytic activity is lost. However, if the EDTA-Ca<sup>2+</sup> 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<sup>2+</sup> to the Ca<sup>2+</sup>-free enzyme.<sup>23</sup>

Inhibitors: Proteinase K is inhibited by DIFP or PMSF (the latter used at final concentration 5 mM).<sup>3</sup> 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 buffered aqueous 40% glycerol solution, containing 10 mM Tris-HCl, pH 7.5, and 1 mM calcium acetate.

Specific activity:  $\geq 500$  units/mL

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

Impurities:

DNase:  $\leq 600$  units/mL

RNase:  $\leq 0.06$  units/mL

Lot specific results for P5568 are reported on the Certificate of Analysis.

### Precautions and Disclaimer

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

### Storage/Stability

The storage temperature for P5568 is 2–8 °C. This product may be stored at –20 °C without detriment.

A Proteinase K solution remains active over a broad pH range (4.0–12.5, optimal pH 8.0) and also over the temperature range of 25–65 °C during use.

### References

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