

# Proteocut K 909L

PK909L

## Product Description

Proteocut K (PK909L) is a subtilisin-type serine protease with a broad substrate specificity that can be used in a wide variety of life science applications, such as to hydrolyse proteins in nucleic acid preparations. The enzyme is completely deactivated at a temperature of 80°C for 6 minutes. Proteocut K (PK909L) has been designed as an alternative to liquid Proteinase K and can be used in the following applications:

Isolation of genomic and plasmid DNA	Improve the cloning of PCR products
Isolation of RNA	NGS and microarray procedures
Removal and inactivation of nucleases and RNases	Protein fingerprinting by mass spectrometry

## Specification

Test	Specification
Appearance (colour)	Light yellow
Appearance (form)	Liquid
Protein (Bradford)	≥ 30 mg/ml
Molecular weight	~27 kDa
Activity	≥ 1,450 U/ml (Haemoglobin assay)
pH Range	6.0 - 10.0
Temperature Range	40 - 70°C
DNase, RNase, Exonuclease activity	Not detectable
Storage Conditions	Store at -20°C. This product will maintain at least 90% of its activity for 12 months.

## Application Performance

The performance of Proteocut K (PK909L) was compared to a commercially available Proteinase K at similar protein concentrations (20 mg/ml liquid). A summary of comparison results can be found below.

Plasmid DNA Extraction	Result Proteocut K (PK909L)	Result Proteinase K
Biotek E.Z.N.A plasmid DNA mini kit used to isolate plasmid DNA from 5ml culture of E.coli DH5α containing a plasmid. 10µl of Proteocut K (PK909L) or 10µl of Proteinase K were added in place of kit protease during DNA isolation	40.6 ng/µl of high-quality plasmid DNA isolated after short incubation time (3-5 minutes) as measured via nanodrop and gel electrophoresis	37.7 ng/µl good quality plasmid DNA isolated after short incubation time (3-5 minutes) as measured via nanodrop and gel electrophoresis
Activity on Restriction Enzymes	Result Proteocut K (PK909L)	Result Proteinase K
Restriction enzymes incubated with 500ng of Proteocut K (PK909L) or 500ng of Proteinase K for 2 hours at 37°C, then 1µg of plasmid DNA added and then incubated for 1 hour at 37°C. Analysis by gel electrophoresis	Confirmed hydrolysis of restriction enzymes by Proteocut K (PK909L) after 2 hours incubation at 37°C; no digestion of vector observed	Confirmed hydrolysis of restriction enzymes by Proteinase K after 2 hours incubation at 37°C; no digestion of vector observed

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## Assay Characteristics

Assay Tests	Result Proteocut K (PK909L)	Result Proteinase K
Protein (Bradford)	≥ 30 mg/ml	20 mg/ml
Protease (Haemoglobin) activity	≥ 1,450 U/ml	≥ 800 U/ml

## General Comparison Summary

Application Tests	Result Proteocut K (PK909L)	Result Proteinase K
Hydrolysis of 5ug Bovine Serum Albumin by 1µg Proteocut K (PK909L) or Proteinase K	>99% Hydrolysis with/without 2% SDS at 37°C in 2 hours	Proteolysis greatly reduced in the presence of SDS
Hydrolysis of 16mg Bacterial Cell Lysate by 1µg Proteocut K (PK909L) or Proteinase K	≥90% Hydrolysis with/without 2% SDS at 37°C in 2 hours	≥90% Hydrolysis with/without 2% SDS at 37°C in 2 hours
Hydrolysis of 20mg CHs5 Fish Cell Lysate by 1µg Proteocut K (PK909L) or Proteinase K	≥95% Hydrolysis with/without 2% SDS at 37°C in 2 hours	<90% Hydrolysis with/without 2% SDS at 37°C in 2 hours
CutSmart® buffer added to 500ng Proteocut K (PK909L) or Proteinase K in 50µl reaction	No change in activity profile as shown with AbCam* assay	No change in activity profile as shown with AbCam* assay
NEB buffer No.3 added to 500ng Proteocut K (PK909L) or Proteinase K in 50µl reaction	No change in activity profile as shown with AbCam* assay	No change in activity profile as shown with AbCam* assay
5% Triton X-100 added to 500ng Proteocut K (PK909L) or Proteinase K	No change in activity profile as shown with AbCam* assay	No change in activity profile as shown with AbCam* assay
5% Tween-20 added to 500ng Proteocut K (PK909L) or Proteinase K	Minimal activity change with AbCam* assay	Minimal activity change with AbCam* assay

\*AbCam Assay – ab112153: Protease Activity Assay Kit (Fluorometric – Red)

## Inactivation Comparison Summary

Deactivation Tests	Result Proteocut K (PK909L)	Result Proteinase K
<b>Reducing Agent TCEP</b> 500ng Proteocut K (PK909L) or Proteinase K incubated with 50mM TCEP at 37°C for 30 mins	100% Inactivation as shown with AbCam* assay	100% Inactivation as shown with AbCam* assay
<b>Inhibitor PMSF</b> 500ng Proteocut K (PK909L) or Proteinase K incubated with 10mM PMSF at 37°C for 30 mins	100% Inhibition as shown with AbCam* assay	100% Inhibition as shown with AbCam* assay
<b>Temperature Inactivation</b> 500ng Proteocut K (PK909L) or Proteinase K incubated at 80°C for 6 mins	100% Inactivation as shown with AbCam* assay	Residual activity detected with AbCam* assay

## Additional Information

All application testing and corresponding results comparing Proteocut K (PK909L) with a commercially available liquid Proteinase K was undertaken by an independent laboratory. This product has not been produced as a food grade product but has been manufactured under ISO 9001 accreditation.