

QUALITY CONTROL FOR MODIFYING ENZYMES

Unit definitions

Unit definition varies from enzyme to enzyme and is given in the product description of this catalogue.

Performance Test

Each enzyme is tested for its performance using tests that are mentioned in product description.

Assay for non-specific endonuclease

All modifying enzymes are checked for the absence of non-specific endonuclease activity by incubating excess of the enzyme with supercoiled plasmid DNA for several hours and determining the level of nicking in supercoiled form. The highest number of units producing unaltered pattern is reported on the data sheet supplied with the enzyme.

Assay for exonuclease activity

The absence of exonuclease activity is checked for all the enzymes by incubating the enzyme with lambda/*Hind* III digest for several hours and running on an agarose gel. The sharp pattern indicates the absence of exonuclease. The highest number of units producing unaltered pattern is reported on the data sheet supplied with the enzyme.

Assay for RNase

The absence of contaminating RNases is tested by incubating total RNA from HeLa cells with excess of enzyme for 4 hours at 37° C and checking for any degradation on gel.

Purification

All modifying enzymes are purified to near homogeneity using the procedures developed at Bangalore GeNei.

Stability

All batches of modifying enzymes are routinely tested for stability by checking unit activity. Most of the enzymes are stable for more than 12 months when stored at -20° C. Exposure to temperatures greater than -20° C should be minimised whenever possible.

Terminal Transferase

Description: The enzyme catalyzes a template-independent addition of dNTPs to the 3'-hydroxyl terminus of DNA molecules. Protruding, recessed or blunt ended double or single stranded DNA molecules serve as a substrate for TdT.

Unit Definition: One unit is the amount of enzyme catalyzing the incorporation of 1 nmol dATP into acid-precipitable material in one hour at 37° C in standard assay conditions in 1 ml reaction volume, using d(pT) as a template.

Application

1. Addition of homopolymer tails to the 3' ends of DNA.
2. Labeling of 3'-ends of DNA

Assay buffer (1X): 50 mM Potassium acetate, 20 mM Tris acetate (pH 7.9), 10 mM Magnesium acetate, 1 mM DTT.

2.5 mM Cobalt chloride solution provided along with 10X Assay Buffer.

Storage buffer: 60 mM KPO₄ (pH 7.2), 150 mM KCl, 1 mM 2-mercaptoethanol, 0.5% Triton X-100 and 50% glycerol.

Store at : -20° C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Terminal Transferase	100 U	105892	MME14	3200

Agarase

Description: Agarase digests agarose by cleaving the agarose sub units to neoagaroligosaccharides. The carbohydrate by-products of the digestion do not interfere with subsequent restriction endonuclease digestion, ligation or transformation of DNA.

Unit Definition: One unit is defined as the amount of enzyme required to digest 200 µl of molten 1% LMP agarose in 1 hour at 42° C.

Application: Purifying DNA or RNA from low melting agarose (LMP).

Assay buffer: (1X) 10 mM Bis-Tris-HCl (pH6.5), 1 mM EDTA.

Buffer supplied at **10X** concentration.

Storage buffer: 50 mM Bis-Tris-HCl (pH 6.5), 1 mM EDTA and 50% glycerol.

Store at : -20° C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Agarase	50 U	105893	MME15	3200

T4 Polynucleotide Kinase

Description: T4 Polynucleotide Kinase catalyses the transfer of gamma phosphate group from ATP to the 5'-hydroxy group of polynucleotides.

Unit Definition: One unit is defined as the amount of enzyme catalysing the production of one nmole of acid insoluble ³²P in 30 minutes at 37° C.

Assay Buffer (1X): 70 mM Tris-HCl (pH 7.6), 10 mM MgCl₂ and 5 mM DTT.

Buffer supplied at **10X** concentration.

Storage Buffer: 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 50 mM KCl, 50% Glycerol and 200 µg/ml Nuclease free BSA.

Application: T4 Polynucleotide Kinase is very useful in the 5' end labeling of nucleic acids.

Performance Test: Labeling and Kination efficiencies are evaluated.

Store at -20° C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
T4 Poly nucleotide Kinase	200 U	105923	MME4S	1650
	1000 U	105922	MME4L	3650

Alkaline Phosphatase (Calf Intestinal Phosphatase) (Molecular Biology Grade)

Description: Alkaline phosphatase molecular biology grade catalyzes the removal of 5' - phosphate groups from DNA, RNA and ribo- and deoxyribonucleoside triphosphates.

Unit Definition: One unit is defined as the amount of enzyme that hydrolyses 1 µmole of p-nitrophenyl phosphate to p-nitrophenol in 1 minute at 37° C in a volume of 1 ml.

Assay buffer (1X) : 50 mM NaCl, 10 mM Tris-HCl (pH 7.9), 10 mM MgCl₂ and 1 mM DTT.

Buffer Supplied at **10X** concentration.

Storage buffer: 50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1 mM MgCl₂, 0.1 mM ZnCl₂ and 50% glycerol.

Application: CIP is used to dephosphorylate vectors in cloning experiments to prevent vector self ligation and to prepare templates for 5' end labeling.

Performance Test: Dephosphorylation efficiency is evaluated.

Store at 4° C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Alkaline Phosphatase (Calf intestinal)	200 U	105930	MME8S	1650
	1000 U	105929	MME8L	5400

Uracil DNA Glycosylase (UDG)

Description: Uracil DNA glycosylase (UDG) excises uracil residues from DNA. It is highly specific in cleaving the glycosidic bond between uracil and deoxyribose sugar of the DNA chain.

Unit Definition: One unit of UDG releases 50 picomoles of uracil from an oligomeric substrate containing single dUMP residue in 15 minutes at 37° C in a 50 µl reaction volume.

Assay Buffer: (1X) 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM DTT, 25 µg/ml BSA.

Buffer Supplied at **10X** concentration.

Storage Buffer: 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 50% glycerol and 100 µg/ml BSA.

Application: UDG is used in a wide variety of applications in molecular biology research e.g., generation of the site directed / nested deletion mutants, study of DNA-Protein interaction, cloning of PCR amplified DNA, elimination of carry over contaminants during PCR, DNA sequencing, etc.

Performance Test: Enzyme tested for efficiency in eliminating carryover contamination during PCR amplification.

Store at -20° C

Note: *E.coli* UDG does not require any cofactors and is active in the presence of EDTA in various buffers but is inhibited by salts (> 50 mM NaCl)

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
UDG	40 U	105886	MME10S	1100
	200 U	105885	MME10L	2450

T4 DNA Ligase

Description: T4 DNA ligase catalyses the linkage of adjacent 5'-phosphate and 3'-hydroxy ends of double stranded DNA by the formation of phosphodiester bond.

Unit Definition: One cohesive end ligase unit is defined as the amount of enzyme required to give 50% ligation of Lambda/*Hind* III digest in 30 minutes at 16° C in 20 µl of the reaction mixture and a 5' DNA termini concentration of 0.12 µM.

Relation to other Ligase Units: One cohesive end Ligase unit approximately equals 0.015 ATP-PP exchange unit (Weiss Unit).

Storage Buffer: 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 50 mM KCl, 1 mM DTT, 50% Glycerol and 200 µg/ml Nuclease free BSA.

Store at - 20° C

Note:

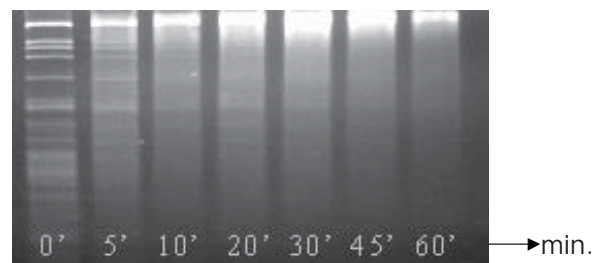
T4 DNA Ligase is supplied with 1 vial each of Cohesive & Blunt end Buffer.

For Buffer Composition refer Buffer Set for Ligation given on page no C62.

Application: This enzyme seals nicks in double stranded DNA and covalently joins DNA fragments with cohesive ends or blunt ends. This enzyme does not act on single stranded DNA.

Performance Test:

1. T4 DNA Ligase is routinely used in-house for quality control checks of all our cohesive and blunt end generating restriction enzymes. It is also used in the **Blue-White standard assay**.
2. Enzyme is tested for its performance in **cloning** experiments.



Blunt End Ligation

Ligation of λ /*Hae* III blunt ended fragments using 1 µl of T4 DNA ligase at 22° C at different time points from 0-60 minutes.

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
T4 DNA Ligase	8000 U	105905	MME1S	1350
	40000 U	105904	MME1L	3650
	100000 U	105903	MME1J	7600
	(High Conc.)	100000 U	105902	MME1HC

Buffer Set for Ligation

Following Buffers can be ordered from us.

Assay buffers:

Cohesive end Ligation Buffer (1X): 50 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, 20 mM DTT, 50 µg/ml Nuclease free BSA and 1 mM ATP.

Buffer supplied at **10X** concentration.

Blunt end ligation Buffer (1X): 50 mM Tris - HCl (pH 7.4), 10 mM MgCl₂, 1 mM DTT, 5% (W/V) Polyethylene glycol-8000 and 1mM ATP.

Buffer supplied in **5X** concentration.

Store at -20°C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Blunt-end Ligation Buffer	0.5 ml x 2	105868	MELB1	1100
Cohesive - end Ligation Buffer	0.5 ml x 2	105869	MELB2	1100
Cohesive & Blunt end Buffer	0.5 ml each	105870	MELB3	1100
Dilution Buffer for T4 DNA Ligase	0.5 ml x 2	105871	MELB4	1100

Test DNA for Ligation

Substrate for T4 DNA Ligase: Lambda/*Hind* III Digest is supplied in **10 mM Tris-HCl (pH 8.0)** and **1 mM EDTA**.

Store at -20°C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Substrate for T4 DNA Ligase	25 µg	105668	MBD18	1100

DNA Polymerase I Klenow Fragment (Sequencing Grade)

Description: The large fragment of DNA polymerase I, also known as the Klenow fragment has two of the three properties of the intact DNA Polymerase I enzyme. The Klenow fragment has 5'-3' DNA polymerase activity and 3'-5' exonuclease activity. However it lacks the 5'-3' exonuclease activity.

Unit Definition:

One unit is defined as the amount of enzyme required to convert 10 nanomoles of total deoxyribonucleotides to an acid insoluble material in 30 minutes at 37° C.

Assay Buffer (1X): 50 mM Tris-HCl (pH 7.6), 10 mM MgCl₂

Buffer supplied at **10X** concentration.

Storage Buffer: 100 mM Tris-HCl (pH 7.5), 10 mM 2-Mercaptoethanol and 50% Glycerol.

Application: Random-primed DNA labeling using random oligonucleotides as primer, for incorporation of non-radioactively labelled and (³²P)-labelled nucleotides.

Filling-in reaction for blunt-end formation of recessed (staggered) ends.

Performance Test:

Enzyme tested for its efficiency in **End Filling** and **random primer labeling** reactions.

Store at **-20° C**

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
DNA Polymerase I (Klenow)	50 U	105921	MME2S	1450
	250 U	105920	MME2L	3200

T7 RNA Polymerase

Description: T7 RNA Polymerase is a DNA dependent RNA polymerase which has high specificity for T7 promoter sequences.

The vectors carrying T7 promoter allow *in vitro* synthesis of defined RNA transcripts from a cloned DNA sequence.

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nanomole ATP into an acid-insoluble form in 1 hour at 37° C.

Assay Buffer: (1X) 40 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 2 mM spermidine and 10 mM DTT.

Buffer supplied at **5X** concentration.

Storage Buffer: 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 20 mM 2-Mercaptoethanol, 100 mM NaCl, 0.1% Triton X-100 and 50% Glycerol.

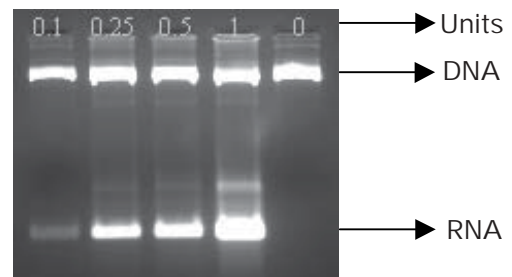
Note: DTT is required for activity and T7 RNA Polymerase is sensitive to salt concentrations. For best result, overall salt concentration should not exceed 50 mM.

Application: T7 RNA polymerase can be used to prepare radiolabelled RNA probes, RNA generation for *in vitro* translation, RNA generation for studies of RNA structure, processing and catalysis.

Performance Test:

Enzyme Tested for its performance in *in vitro* transcription assays.

Store at **-20° C**



***In vitro* Transcription:** 1 µg of control template DNA incubated with 0.1, 0.25, 0.5 and 1U of T7 RNA Polymerase, under standard assay conditions for 1 hour at 37° C. The reaction mixture was then analysed on 2% agarose gel.

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
T7 RNA Polymerase	1000 U	105928	MME6S	1350
	5000 U	105927	MME6L	3100

AMV Reverse Transcriptase

Description: AMV Reverse Transcriptase is a RNA - dependent DNA polymerase purified from Avian Myeloblastosis virus. The enzyme is used extensively in cDNA synthesis and dideoxy DNA sequencing.

The enzyme possesses several enzymatic activities which include an RNA directed DNA synthesis, a DNA-dependent DNA polymerase, an RNase H activity and an unwinding activity.

Unit definition: One unit of AMV reverse transcriptase is defined as amount of enzyme which incorporates 1 nanomole of (³H) dTMP into an acid insoluble product in 10 minutes at 37° C using poly (A) - oligo (dT) as template - primer.

Assay Buffer: (1X) 50 mM Tris-HCl (pH 8.5), 8 mM MgCl₂, 30 mM KCl and 1mM DTT.

Buffer supplied in **5X** concentration.

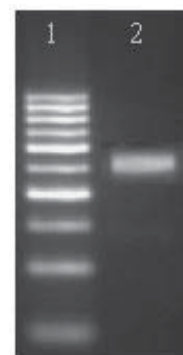
Storage buffer: 200 mM Phosphate Buffer (pH 7.2), 2 mM DTT, 0.2% Triton X-100 and 50% glycerol.

Application: The enzyme is used extensively in cDNA Synthesis and dideoxy DNA sequencing.

Performance Test:

Enzyme Tested for its performance in RT-PCR reactions.

Store at -20° C



Lane 1: 100 bp DNA ladder

Lane 2: RT-PCR product of 500 bp fragment from G3PDH gene (cDNA from total HeLa RNA)

RT PCR

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
AMV Reverse Transcriptase	100 U	105931	MME9L	3200

M-MuLV Reverse Transcriptase

Description : M-MuLV RT is a RNA dependent DNA polymerase that uses single stranded RNA or DNA as template. It lacks endonuclease activity and has lower RNase H activity.

Unit definition: One unit of M-MuLV reverse transcriptase is defined as amount of enzyme which incorporates 1 nanomole of (³H) dTMP into an acid insoluble product in 10 minutes at 37° C using poly (A) - oligo (dT) as template - primer.

Assay Buffer: (1X) 50mM Tris-HCl (pH 8.5), 8 mM MgCl₂, 30 mM KCl and 1mM DTT.

Buffer supplied in **5X** concentration.

Storage Buffer : 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% Igepal and 50% glycerol.

Application: The enzyme is used in cDNA synthesis and dideoxy DNA sequencing.

Performance Test:

Enzyme Tested for its performance in RT-PCR reactions.

Store at: - 20° C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
M-MuLV RT	100 U	105889	MME13	1100
	1000 U	105891	MME13S	2750
	5000 U	105890	MME13L	5500

Uracil DNA Glycosylase Inhibitor (UGI)

Description: Uracil DNA glycosylase inhibitor (UGI) is a *B. subtilis* phage PBS2 encoded low molecular weight, thermostable acidic protein. UGI forms a tight complex with UDG in a 1:1 stoichiometry and potently inhibits UDG.

Unit definition: One unit of UGI completely inactivates one unit of UDG under suitable assay conditions.

Reaction Buffer: UGI forms a tight complex with *E. coli* UDG under varied buffer conditions and inhibits UDG activity.

Storage Buffer: 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 50% glycerol and 100 µg/ml BSA.

Store at -20° C

Application: UGI is used to ensure complete inhibition of UDG.

Note: UDG is active in presence of EDTA and may remain partially active even after terminating the reactions by heating at 95° C. UGI itself is highly thermostable and retains full activity even after keeping in boiling water for 10 minutes.

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
UGI	40 U	105888	MME11S	1100
	200 U	105887	MME11L	2200

Human Placental RNase Inhibitor

Human Placental RNase Inhibitor is an acidic protein of molecular weight near 50 kDa. It forms 1:1 complex with RNase A and is a noncompetitive inhibitor of this enzyme. RNase Inhibitor is active over a broad pH range and requires DTT for its activity.

Unit definition: One unit is defined as the amount of RNase Inhibitor required to inhibit the activity of 5 ng of RNase A by 50%.

Storage buffer: 20 mM HEPES-KOH (pH 7.6), 50 mM KCl, 5 mM DTT and 50% glycerol.

Application: RNase inhibitor is used to protect the mRNA in cDNA synthesis, in *in vitro* transcription /translation system and *in vitro* RNA synthesis.

Note: 5 mM DTT concentration is critical for the inhibitor and so has to be maintained during long and repeated uses.

Store at - 20° C

Performance Test: Used extensively in our lab for *in vitro* transcription assays and RT-PCRs.

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Human Placental RNase inhibitor	1000 U	105389	FC11	3200

RNase H

Description: Ribonuclease H (RNase H) is an endoribonuclease that specifically hydrolyzes the phosphodiester bonds of RNA which is hybridized to DNA. The enzyme does not digest single or double stranded DNA.

Source: A recombinant *E. coli* strain that carries a plasmid containing the cloned RNase H gene (*rnh*) from *E. coli*.

Unit definition: One unit is defined as the amount of enzyme that will hydrolyze 1 nmol of the RNA in [³H]-labeled poly (dA) • poly (dT), to acid-soluble ribonucleotides in a total reaction volume of 50 µl in 20 minutes at 37° C.

Assay buffer: 75 mM KCl, 50 mM Tris-HCl (pH 8.3), 3 mM MgCl₂, 10 mM dithiothreitol.

Storage buffer: 100 mM KCl, 20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 0.1 mM EDTA, 0.1mM dithiothreitol, 50 µg/ml BSA and 50% glycerol.

Application:

- Removal of Poly (A) tails of mRNA hybridized to Poly (dT).
- Hydrolysis of mRNA following reverse transcription reaction during second strand cDNA synthesis.
- Oligo deoxyribonucleotide mediated degradation of RNA.

Store at - 20° C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
RNase H	100 U	105446	FC54	2200

RNase A (Ribonuclease A)

Ribonuclease A (bovine pancreas) supplied as white lyophilized powder which dissolves readily at concentration of 2 mg/ml in analytical grade water to give a clear colourless solution.

Purity: > 70% pure as checked by ion exchange chromatography.

Activity: In the range of 70 - 80 U/mg protein.

Enzyme is not tested for DNase activity.

Unit definition: One unit is that amount of enzyme causing hydrolysis of RNA at a rate such that k (velocity constant) equals unity at 25° C and pH 5.0.

Store at - 20° C

Application:

- Used in phage extraction

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
RNase A	50 mg	105425	FC34S	1600
	250 mg	105424	FC34L	4900

RNase A (Ribonuclease A) (DNase Free)

Ribonuclease A (bovine pancreas) is used to hydrolyse RNA during isolation of DNA. Supplied as white lyophilized powder which dissolves readily at concentration of 2 mg/ml in analytical grade water to give a clear colourless solution.

Essentially protease free and > 95% pure as checked by ion exchange chromatography.

Activity: In the range of 80 - 90 u/mg protein.

Unit definition: One unit is that amount of enzyme causing hydrolysis of RNA at a rate such that k (velocity constant) equals unity at 25° C and pH 5.0.

Store at - 20° C

Application:

- In the isolation of DNA for RNA-free DNA.
- As a molecular weight marker.

Performance Test: Efficiency tested in plasmid preparations by alkali lysis.

DNase is not detected

Absence of DNase contamination: 1 µg of pBR 322 plasmid DNA was incubated with RNase, at a final concentration of 20 µg/ml, for 30 minutes at 37° C in a 50 µl reaction volume. No nicking or degradation of plasmid DNA observed on running the sample along with controls on a 1.2% agarose gel.

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
RNase A (DNase Free)	10 mg	105414	FC25S	1000
	50 mg	105413	FC25L	3000
	100 mg	105412	FC25J	5000

DNase I (RNase Free)

Description: DNase I (bovine pancreas) is a glycoprotein and double strand specific endonuclease which is made free of RNase activity for specific applications. The protein requires divalent cations for maximal activity. Supplied in 20 mM Tris-HCl (pH 7.6), 1mM DTT, 0.1 mg/ml Nuclease Free BSA and 50% (v/v) glycerol.

Unit Definition: One unit is that amount of enzyme causing an increase in absorbance at 260 nm by 0.001 per minute at 25° C and pH 5.0.

Application:

1. DNase I (RNase free) is used in purification of DNA- free RNA made by in-vitro synthesis using SP6 or T7 RNA Polymerase system.
2. Used for radioactive labeling by nicktranslation

Storage: -20° C.

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
DNase I (RNase Free)	1000 U	105448	FC56	5500

DNase I (Deoxyribonuclease I)

Description: Supplied as white lyophilised powder which dissolves readily at 5 mg/ml in 0.15 M sodium chloride to give a clear colourless solution. The enzyme loses its activity irreversibly by heat treatment at 80° C for 10 min.

Activity: In the range of 1000 u/mg material (Kunitz).

Unit definition: One unit is that amount of enzyme causing an increase in absorbance at 260 nm by 0.001 per min. at 25° C and pH 5.0.

Store at - 20° C

Application: Deoxyribonuclease I (bovine pancreas) is used to catalyze random degradation of both single and double-stranded DNA producing 5'-P terminal oligonucleotides.

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Deoxyribo-nuclease I	10 mg	105418	FC28S	1200
	50 mg	105417	FC28L	3800

Proteinase K

Proteinase K is a broad spectrum serine protease useful for general digestion of proteins. It is active in presence of SDS, EDTA or Urea and has a pH - optimum of 7.5 - 10.5.

Application: Degradation of proteins during DNA and RNA isolation.

Unit definition: One unit of proteinase K produces 1 μ mole of Folin-positive amino acid in 1 min at 37° C. Supplied as lyophilized powder.

Store at -20° C

Ordering Information:

Product	Size	New Cat #	Old Cat #	Price (Rs)
Proteinase K	25 mg	105973	PK1S	1400
	100 mg	105972	PK1L	4200
	1 g	105971	PK1B	39000