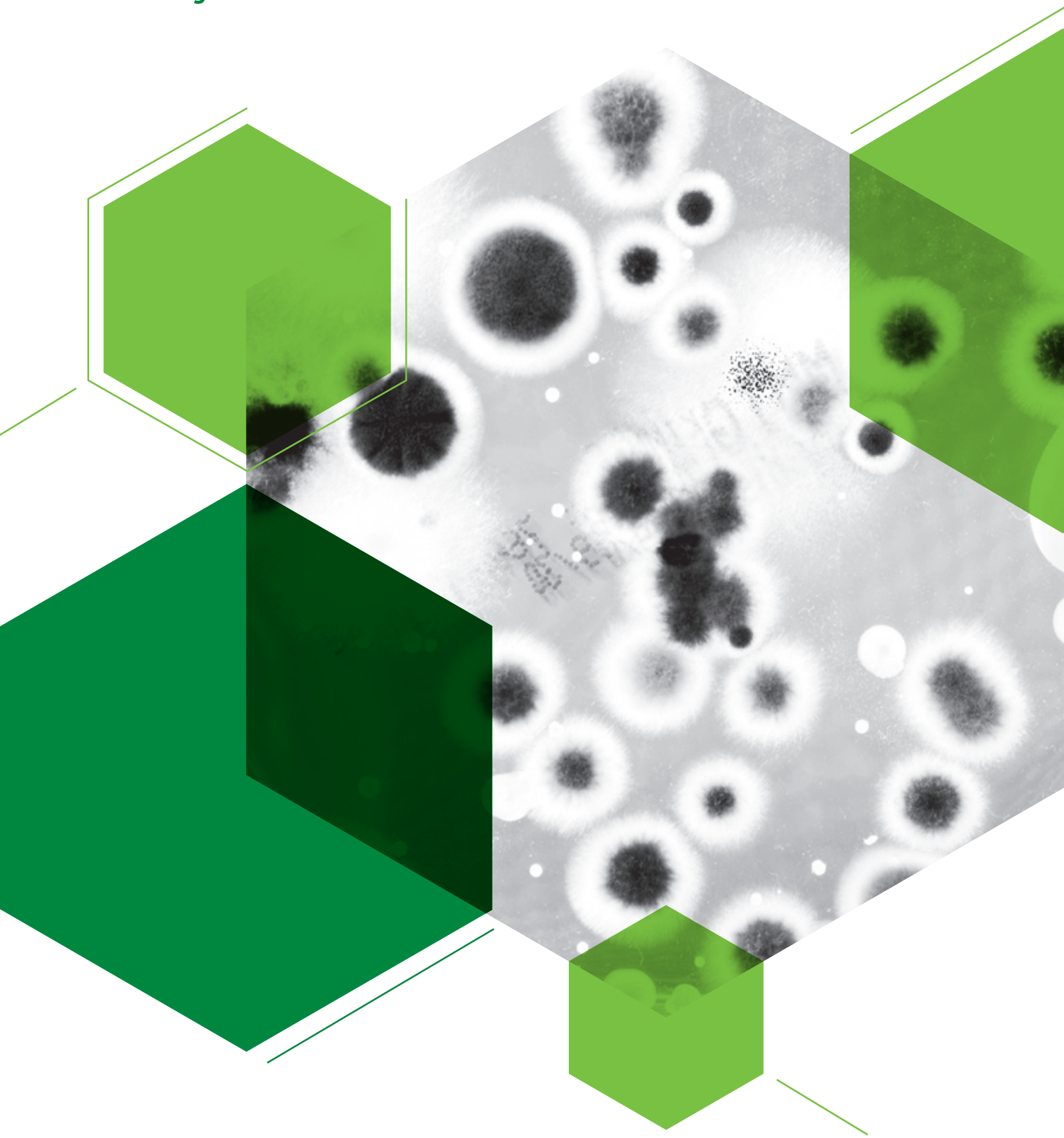
Enzymes & Proteins



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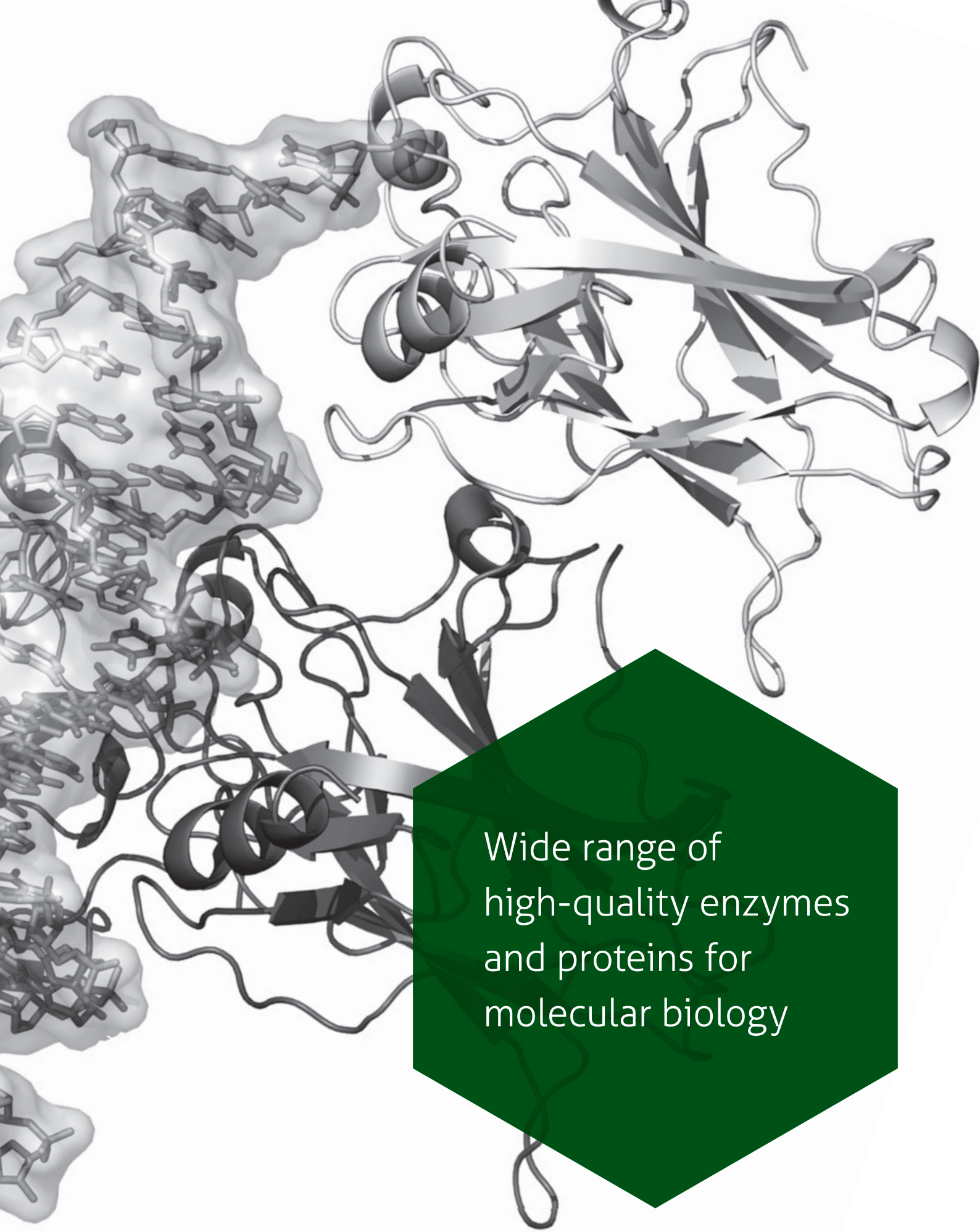

Wide range of high-quality enzymes and proteins for molecular biology

ENZYMES & PROTEINS

We offer a wide range of high-quality enzymes and proteins for molecular biology including proteases, ribonucleases, reverse transcriptases, DNA ligases, thermostable polymerases and single-stranded DNA binding proteins.

Proteinase K

A recombinant Proteinase K from *Tritirachium album* belongs to the group of subtilisine-related serine proteases. It is an endopeptidase of low substrate specificity and high specific activity. It's widely used for DNase and RNase removal during DNA and RNA extraction. It is also used for the albumin impurities removal, increasing cloning efficiency and determination of enzyme localization.

- Specific activity: 30 U/mg
- Exhibits stability in a wide pH (4-12) and temperature range (4 -37°C), optimal activity at pH=7.5-8.0 and 37°C
- It is not inactivated by chelating agents (e.g. EDTA), chaotropic salts, detergents (e.g. SDS, urea) or trypsin-specific inhibitors
- Exhibits prolonged stability due to the presence of calcium ions (Ca^{2+}), which protects enzyme from autolysis

TEV Protease

An engineered form of cysteine protease from Tobacco Etch Virus (TEV) is mainly used for removing affinity markers from fusion proteins, N-terminal poli-HIS and C-terminal poli-ARG. It has been genetically modified in order to increase activity and autolysis protection. The TEV Protease recognises an amino acid sequence: E-X-X-Y-X-Q-(G/S)(2-5) (where X is any amino acid residue). The digestion occurs between Q and G/S.

The most common sequences are ENLYFQG or ENLYFQS. The TEV Protease is not inactivated by the serine or cysteine protease inhibitors.

- concentration of 5 U/µl
- 10 U of TEV Protease cleaves >95% of 20 µg of a test fusion protein in 2 hours at 30°C

Lysostaphin

Lysostaphin is an enzyme which has an ability to hydrolyse cell walls of the bacteria from a *Staphylococcus* genus. The lysostaphin is an endopeptidase which specifically cleaves the pentaglycine cross bridges found in the staphylococcal peptidoglycan.

The lysostaphin is widely used for obtaining protoplasts and also for isolation of enzymes and cell structures from a genetic material from *Staphylococcus* bacteria.

- Specific activity: 400 U/mL
- Concentration: 1 mg/mL

Thermostable Single-Stranded DNA Binding Proteins (SSB)

TaqSSB – PCR enhancer

Thermostable SSB protein isolated from *Thermus aquaticus*, recommended for general use with techniques requiring extremely high temperature conditions, such as nucleic acid amplification (PCR) and DNA sequencing.

Extreme Thermostable SSB proteins (TneSSB, TmaSSB)

Extreme Thermostable Single-Stranded DNA Binding proteins isolated from the hyperthermophilic bacteria *Thermotoga neapolitana* (TneSSB) and *Thermotoga maritima* (TmaSSB). Due to the extreme thermostability, TneSSB and TmaSSB can be used in molecular biology applications that require extremely high temperature conditions, such as nucleic acid amplification and sequencing.

Thermostability
TneSSB is the most thermostable SSB protein identified to date, with a melting temperature (Tm) of 112.5°C (Tm of TmaSSB is 109.3°C and of TaqSSB is 86.8°C). The half-live of the ssDNA-binding activity at 100°C is 12 h (TmaSSB is 10 h).

Remaining SSB proteins (from *Thermus thermophiles*, *Deinocossus radiopugnans*, *D. geothermalis*, *D. murrai*) are produced upon request.

Applications of thermostable SSB proteins:
- Prevents PCR inhibition
- Increases amplification efficiency
- Increases selectivity and specificity of multiplex PCR
- Protects single stranded DNA from degradation
- Reduces secondary structure formation, which inhibits PCR
- Enhances amplification of difficult templates (e.g. rich in GC)
- Stimulates fidelity and processivity of *Taq* polymerase
- Reacts with RNA allowing increase of a synthesized cDNA size
- Stabilises ssDNA in the site-specific mutagenesis
- Facilitate in obtaining complete digestion by restriction endonucleases

We put emphasis on the selection and optimization of biosynthesis, purification and formulation process conditions.

T4 DNA Ligase

T4 DNA Ligase is a recombinant enzyme purified from *Escherichia coli*. T4 DNA ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphoryl and 3'-hydroxyl termini in duplex DNA or RNA. The enzyme joins blunt end and cohesive end termini as well as repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

Applications
- → Cloning of restriction fragments and PCR amplification products
- → Joining of double-stranded oligonucleotide linkers and adaptors to DNA
- → Site-directed mutagenesis
- → Ligation Mediated PCR e.g. Amplified fragment length polymorphism (AFLP)
- → Sealing nicks in double-stranded DNA
- → Recircularization of linear DNA.

Components
- → T4 DNA Ligase
- → 10x Ligation Buffer
- → ATP Solution

Concentration
- → 5 U/µl

Quick Ligase

Quick Ligase is designed for the efficient ligation of cohesive end or blunt end DNA fragments in 5 and 15 minutes respectively at room temperature 25°C. Rapid ligation is based on the combination of T4 DNA Ligase with a unique 2x Quick Ligation Buffer.

Features
- → Dramatically decreases time for DNA cloning
- → Rapid 5 to 15 minute protocol at room temperature
- → Efficient and reliable ligations of cohesive and blunt-ended DNA
- → No loss of transformation efficiency

TRANSCRIPT*ME* – Reverse Transcriptase

TRANSCIRPT*ME* is a modified, recombinant form of the Reverse Transcriptase from the Moloney Murine Leukemia Virus (M-MuLV) purified from *Escherichia coli*. The enzyme has been modified in order to promote stability. The ***TRANSCIRPT****ME* synthesizes the complementary DNA strand in the presence of a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template. It lacks 3'→5' exonuclease and RNase H activity, which improves synthesis of full-length cDNA even for long mRNA, using random priming. The enzyme gives high yields of first strand cDNA up to 10 kb long.

The increased thermostability of the ***TRANSCRIPT****ME* allows the processing of the reaction at a higher temperature (optimum activity at 50°C), which may increase efficiency and specificity of the transcribed RNA regions, which are rich in GC pairs and/or contain secondary structures.

Concentration: 200 U/µl

Features
- → High yields of full-length cDNA synthesis (up to 10 kb long)
- → Maintains the RNA- and DNA-dependent DNA polymerase activities
- → Formulated to increase sensitivity in RT-qPCR and RT-PCR assays
- → Starting material: 10 pg – 5 µg of total RNA or 10 pg – 500 ng of mRNA
- → Optimal reaction temperature: 50°C
- → Increased thermostability
- → Lacks RNase H and 3'→5' exonuclease activities
- → Suitable for the amplification of difficult RNA templates

Applications
- → Full-length cDNA synthesis for use in RT-qPCR and two-step RT-PCR assays
- → cDNA synthesis for molecular cloning
- → cDNA library construction
- → RNA analysis

*RIBO*PROTECT – RNase Inhibitor

RIBO*PROTECT* RNase Inhibitor is a recombinant inhibitor of pancreatic ribonucleases, such as RNase A, RNase B and RNase C, purified from *Escherichia coli*. This protein is useful in any application where eukaryotic RNase contamination is a potential problem. This inhibitor can be used to protect RNA template in cDNA synthesis or *in vitro* transcription/translation reactions. ***RIBO****PROTECT* is not effective against RNase 1, RNase T1, RNase T2, S1 nuclease, RNase H or RNase from *Aspergillus sp.*

Applications

- → cDNA synthesis, RT-PCR and RT-qPCR
- → *in vitro* transcription/translation
- → RNA extraction and purification

Concentration: 40 U/µl

RNase A (DNase-free)

The Ribonuclease A (RNase A) is an endoribonuclease, that selectively cleaves single-stranded RNA 3' next to pyrimidine residues (cytosine, uracil). It degrades RNA to cyclic nucleotide monophosphates leaving a 5'-OH and 2'-, 3'-cyclic monophosphate. The enzyme exhibits no endonuclease or exonuclease activity towards DNA substrates. The RNase A is used to remove RNA during the isolation procedures of plasmid and genomic DNA.

Applications

- → RNA protection assays
- → Purification of RNA-free DNA
- → Plasmid and genomic DNA isolation
- → Removal of RNA during recombinant proteins preparations

Activity: 90 U/mg (Kunitz)

Thermostable DNA Polymerases

Polymerases for routine PCR applications are described in the „PCR Reagents" brochure.

PRODUCT	SIZE	CAT. NO.
Proteinase K	50 mg	RP10B
	250 mg	RP101B
	1000 mg	RP102B
TEV Protease	1000 U	RP171
	10.000 U	RP172
Lysostaphin	400 U	RP12
	2000 U	RP125
TaqSSB	50 µg	RP30
	250 µg	RP305
TneSSB	50 µg	RP31-050
	250 µg	RP31-250
TmaSSB	50 µg	RP32-050
	250 µg	RP32-250
T4 DNA Ligase	500 U	EN11-050
	2500 U	EN11-250
Quick Ligase	50 reactions	EN12-050
	150 reactions	EN12-150
TRANSCRIPT*ME* Reverse Transcriptase	10.000 U	RT32-010
	50.000 U	RT32-050
RIBO*PROTECT* RNase Inhibitor	2000 U	RT33-020
	10.000 U	RT33-100
RNase H	250 U	RT34-025
	1250 U	RT34-125
RNase A	50 mg	RP145
	250 mg	RP148

We offer protein production services from gene synthesis to purification and formulation performed in modern research facilities by experienced scientific staff.

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