

Cloning & Enzymes

1999, Dr. Victor E. Velculescu

Tantalizing transcriptomes—SAGE and its use in global gene expression analysis

Dr. Velculescu received the grand prize for his essay which discusses his doctoral research on the development of SAGE (serial analysis of gene expression) and its application to unraveling the differences in gene expression between normal cells and tumor cells..

Dr. Velculescu earned his bachelor's degree in the Department of Biological Sciences at Stanford University. He later attended the Johns Hopkins University School of Medicine where he was awarded his PhD in the Program of Human Genetics and Molecular Biology in 1998 and his MD in 1999. His doctoral work on the development and application of SAGE to analyze gene expression patterns was performed in the laboratory of Ken Kinzler at the Johns Hopkins Oncology Center.

GE & Science Prize for Young Life Scientists is supported by GE Healthcare and the journal Science, which is published by AAAS, the nonprofit society. Used with permission of AAAS © 2007.

Further information on how to enter, plus past winners and their essays can be found at:

www.gelifesciences.com/science



Chapter 4

Bulk and Custom Orders	132
Kits for cDNA Synthesis	133
Products for PCR and cDNA Cloning	
Cloning Kits	135
Transfection	135
Vectors	136
General Cloning Vectors	137
Cloning Products	137
Oligonucleotides	
Oligonucleotides for Use as Primers	137
Polynucleotides	
Polynucleotide Nomenclature	138
Major Polynucleotide Products	138
Polynucleotides Listed by Category	139
Natural DNAs	
Modifying Enzymes	139
Alkaline Phosphatases	140
Kinases	140
Ligases	141
Nucleases	141
Polymerases	142
Proteases	143

made2measure

Products in this chapter can be customized to your precise requirements, including small-scale packs, concentrations, special blends, specific QC testing, and scale-up.

In addition, we offer a "Room-Temperature Stable" reagent development and manufacturing service where we develop your -20°C reagents and kits for storage and shipping at room temperature.

Our manufacturing standards are certified to ISO 9001:2000 with Six Sigma implementation throughout the manufacturing process.

Contact us for more information at made2measure@ge.com, and visit us on the web at www.gelifesciences.com/custom.

To ensure that we handle your inquiry efficiently, please provide the product name, catalog code, and volume requirements.

Custom Reagents

Our custom products are tailored to your precise requirements to deliver specifications you can rely on with lot-to-lot consistency. Most of our products can be customized to meet various needs including small-scale packs, changes in concentration, special blends, specific QC testing, and scale-up.

In addition, we offer a "Room-Temperature Stable" reagent development and manufacturing service. This means that your -20°C reagents and kits can be designed and manufactured for storage and shipping at room temperature.

Our manufacturing standards are certified to ISO 9001:2000 with Six Sigma implementation throughout the entire manufacturing process.

Contact us for more information at made2measure@ge.com, visit us on the web at www.gelifesciences.com/custom.

To ensure that we handle your inquiry as efficiently as possible, please provide the product name, catalog code, and the quantity required. If you need a customized product, please provide our product name and code with your specifications and volume requirements.



All stages of production are controlled and monitored by the latest analytical instrumentation, and all custom products are tested and refined to meet high quality standards and batch-to-batch reproducibility.

First-Strand cDNA Synthesis Kit

- For synthesis of single-stranded cDNA prior to amplification by PCR.
- Features preassembled bulk reaction mixes to save time and minimize potential for pipetting errors.
- Allows users to choose primers.
- Includes a choice of two primers: pd(N)₆ random hexamers and NotI-(dT)₁₈.

First-Strand cDNA Synthesis Kit is designed to generate full-length first-strand cDNA from mRNA templates. Following first-strand cDNA synthesis, the sample can be used directly for *in vitro* amplification using PCR or for second-strand synthesis using the Gubler-Hoffman method. Primers are not included in the preassembled First-Strand Reaction Mixes, thus allowing use of a primer of choice. Two primers are included with the kit. pd(N)₆ Primer (random hexamer) is used to prepare libraries for screening with antibodies, to increase the representation of 5'-ends of mRNAs having significant secondary structure, or to copy mRNAs lacking a poly(A) tail. The NotI-(dT)₁₈ Bifunctional Primer can be used to prime selectively on mRNA having a poly(A) tail. It can also be used as a PCR primer following first-strand synthesis. The NotI restriction site, located at the 5'-end of the primer, is particularly useful for subsequent directional cloning of cDNA and PCR products.

ORDERING INFORMATION

Product	Quantity	Code Number
First-Strand cDNA Synthesis Kit	55 reactions	27-9261-01

For pricing information, visit www.gelifesciences.com/orderonline

Related Products	Code Number	Refer To
illustra QuickPrep Micro mRNA Purification Kit		page 171

First-Strand cDNA Synthesis Kit contains the following reagents, sufficient for up to 55 first-strand syntheses, each of which will produce enough material for up to 15 amplification reactions: Bulk First-Strand cDNA Reaction Mixes, mRNA Standard, DTT Solution, RNase-Free Water, pd(N)₆ Primer, NotI-(dT)₁₈ Bifunctional Primer and instruction booklet.

Ready-To-Go You-Prime First-Strand Beads

- For synthesis of first-strand cDNA templates from total RNA or polyadenylated RNA—using a primer of choice—for RT-PCR.
- Predispensed, ambient-temperature-stable, single-dose reaction beads minimize pipetting errors, avoid cross-contamination, and ensure optimal performance.
- First-strand reaction beads contain no primer, thus allowing users to select a first-strand primer of choice.
- Ideal for research applications that use PCR to detect and quantify eukaryotic RNA from a variety of samples.
- Reaction beads are function-tested for first-strand synthesis of cDNA up to 7.5 kb and in RT-PCR from blood samples.
- Completed reactions (33 μ l final volume) can be used directly in PCR after adding water, *Taq* DNA polymerase and primers; first-strand cDNA can also be used as a template for traditional Gubler-Hoffman second-strand cDNA synthesis.

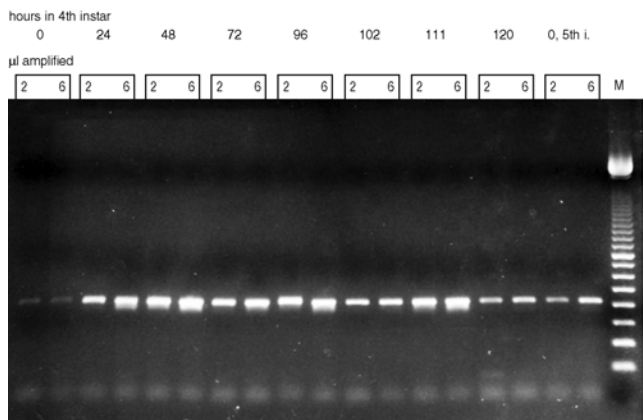
The kit provides 50 preformulated, single-dose reaction beads prepackaged in thin-walled 0.5 ml tubes compatible with most thermal cyclers. Each reaction contains: Cloned FPLCpure M-MuLV Reverse Transcriptase, RNase Inhibitor and Nucleotides, First-Strand Reaction Mix Beads, Control Mix Beads and instruction booklet. (Five Control Mix Beads containing globin mRNA and two globin-specific PCR primers are included for evaluating the performance of the Reaction Mix Beads and the PCR amplification).

ORDERING INFORMATION

Product	Quantity	Code Number
Ready-To-Go You-Prime First-Strand Beads	50 reactions	27-9264-01

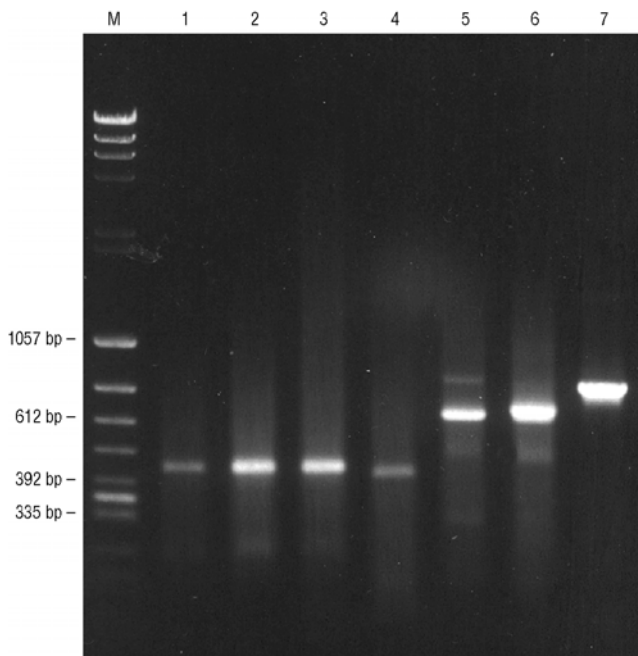
For pricing information, visit www.gelifesciences.com/orderonline

Related Products	Refer To
illustra RNAspin Midi Kit	page 169
illustra QuickPrep Micro mRNA Purification Kit	page 171



RT-PCR determination of the relative levels of mRNA for haemolymph juvenile-hormone-binding protein in the black larval mutant of *Manduca sexta* (tobacco hornworm). QuickPrep Micro mRNA Purification Kit (27-9255-01) was used to isolate mRNA from < 30 mg of fat body dissected from staged fourth instar larvae. 50 ng of mRNA were converted to first-strand cDNA using Ready-To-Go You-Prime First-Strand Beads. Samples (2 μ l and 6 μ l) of a 1:10 dilution of the cDNA product in water were amplified by PCR in a 50 μ l reaction. The cycling parameters were 95°C for 5 min followed by 27 cycles of 95°C, 1 min; 60°C, 1 min; 72°C, 1 min. 10 μ l of each reaction was loaded onto a 1.5% agarose gel. 0, 5th i = 0 h, fifth instar; M = 100 Base-Pair Ladder (27-4007-01). Samples courtesy of Walter Goodman and Tony Orth, Department of Entomology, University of Wisconsin, Madison, WI, USA.

Ready-To-Go T-Primed First-Strand Kit



RT-PCR products from various sources. In each case, first-strand cDNA was synthesized from animal liver mRNA using Ready-To-Go T-Primed First-Strand Kit and amplified by PCR. Lane 1, β -actin gene from 500 pg of sheep liver mRNA; lane 2, β -actin from 1.0 μ g of sheep liver mRNA; lane 3, β -actin from 500 pg of hog liver mRNA; lane 4, α -globin from 500 pg of hog liver mRNA; lane 5, RNase inhibitor from 1.0 μ g of rabbit liver mRNA; lane 6, RNase inhibitor from 1.0 μ g of sheep liver mRNA; lane 7, RNase inhibitor from 1.0 μ g of hog liver mRNA. M = λ DNA-HindIII/ ϕ X-174 RF DNA-HincII (27-4052-01) (see page 321).

ORDERING INFORMATION		
Product	Quantity	Code Number
Ready-To-Go T-Primed First-Strand Kit	50 reactions	27-9263-01

For pricing information, visit www.gelifesciences.com/orderonline

Related Products	Refer To
illustra RNAspin Midi Kit	page 169
illustra QuickPrep Micro mRNA Purification Kit	page 171

- For synthesis of full-length first-strand cDNA templates from polyadenylated RNA for use in RT-PCR.
- Preformulated, ambient-temperature-stable, single-dose glassified reaction mixes minimize pipetting errors, avoid potential cross-contamination of subsequent PCR products and ensure optimal performance from each reaction.
- Predispensed reaction mixes are ideal for research applications which use PCR to detect and quantify eukaryotic RNA species isolated from a variety of cell sources and tissue samples.
- Reaction mixes include NotI-(dT)₁₈ primer in sufficient quantity to serve as a downstream PCR primer for performing 3'-RACE. Final reaction volume is 33 μ l once reconstituted.

The kit provides 50 single-dose, ambient-temperature-stable, first-strand cDNA synthesis reactions, each containing NotI-(dT)₁₈ Primer, Cloned FPLCpure M-MuLV Reverse Transcriptase, RNase Inhibitor, and Nucleotides. (A reaction mixture containing rabbit globin mRNA and specific globin primers is included as a control).

Amersham CyScribe First-Strand cDNA Labeling Kit

For main product entry, see page 338.

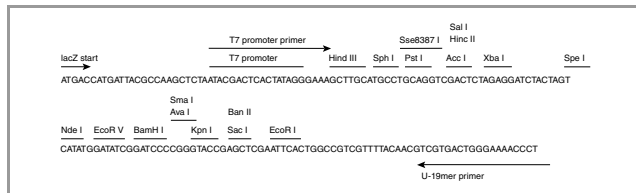
Amersham CyScribe Post-Labeling Kit

For main product entry, see page 339.

Blunt-ended PCR Cloning Kit

- **Optimized for the rapid and efficient cloning of all PCR products with blunt or sticky ends.**
- Replaces traditional TA cloning approaches by offering consistently better signal-to-noise ratios and as many as 25-fold more positive clones.
- Amplicons generated by proof-reading thermostable polymerases are fully compatible.
- High efficiency cloning, no requirement for time-consuming restriction digests and blue/white screening.
- Linearized at the EcoRV site.

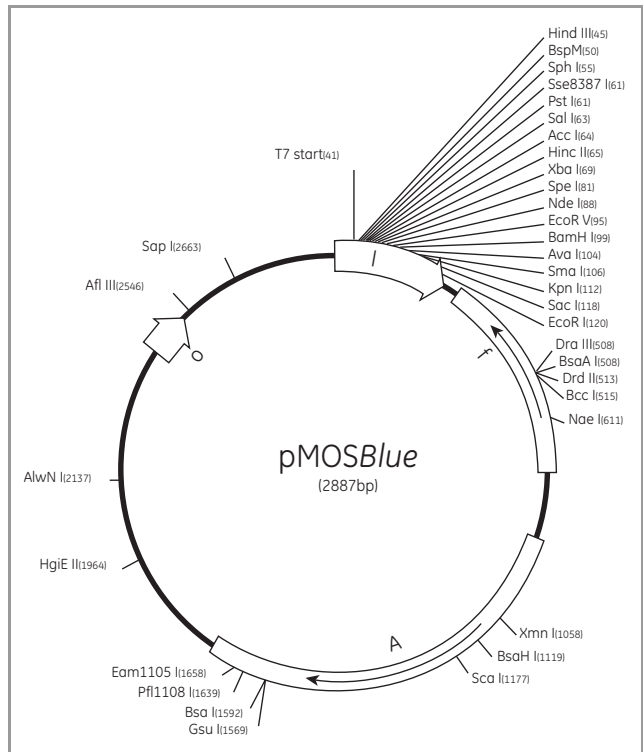
Components of the kit, sufficient for 40 ligations and transformations and to be stored at -70°C, include: pMOSBlue dephosphorylated blunt vector, positive control insert, 10x PK buffer, PK enzyme mix, T4 DNA Ligase, DTT, nuclease-free water, pMOSBlue Competent Cells, SOC medium and test plasmid for transformation.



pMOSBlue multiple cloning region.

ORDERING INFORMATION		
Product	Quantity	Code Number
Blunt-Ended PCR Cloning Kit	40 reactions	RPN5110

For pricing information, visit www.gelifesciences.com/orderonline



CellPfect Transfection Kit

- **For DNA transfection of eukaryotic cells with high efficiency.**
- Includes protocols and optimized buffers for calcium phosphate transfection for stable gene expression, and DEAE Dextran solution for transient expression studies.
- Contains sufficient reagents for 25 to 100 transfections.

ORDERING INFORMATION		
Product	Quantity	Code Number
CellPfect Transfection Kit	1 kit	27-9268-01

For pricing information, visit www.gelifesciences.com/orderonline

Expression and Fusion Vectors

SELECTION GUIDE – Vectors			
Feature/Application	Prokaryotic Gene Fusion Vectors		General Cloning Vectors
	GST Gene Fusion Vectors (see page 431)	pEZZ 18 (see page 450)	M13 Vectors (see page 137)
Selectable Marker(s)	Amp	Amp	
Blue/White Screening		X	X
MCS (# unique sites)	vector-dependent	10	10
f1 Origin			X
<i>in vitro</i> Transcription			
Prokaryotic Expression	X	X	
Fusion Partner	GST	protein A	
Protease Cleavage Sites	X		
Promotor	<i>tac</i>	<i>spa lacUV5</i>	<i>lac</i>
Induction	IPTG		IPTG
RBS	X	X	
ATG	X	X	
Transcription Termination			
Translation Termination	X		
Eukaryotic Expression			
Splicing/Polyadenylation			
Promotor Analysis			
Gene Cartridge			
cDNA Cloning			
Host Strains	<i>E coli</i>	<i>E coli</i>	<i>E coli F'</i>
Common Restriction Sites (# MCS Sites, where applicable/ Total # Sites in Plasmid)	BL21 (included)	β-gal, α-acceptor	
AccI	*		1/1
ApaI	0/1	0	0
AvaI	*		0/2
BamHI			1/1
EagI	*	0/1	0
EcoRI			1/1
HincII	*		1/1
HindIII	0	1/1	1/1
HpaI	0/1	0	0
KpnI	0	1/1	1/1
MluI	0/1	0/2	0
NcoI	0	0	0
NheI	0	0/1	0
NotI	*	0/1	0
PstI	0/1	1/1	1/1
SacI	0	1/1	1/1
SacII	0	0	0
Sall	*	1/1	1/1
SfiI	0	0	0
SmaI	*	1/1	1/1
SphI	0	1/1	1/1
SspI	0/2	0/5	0/6
XbaI	0	1/1	1/1
XhoI	*	0	0
XmaI	*	1/1	1/1

Note: For high-level transformation of host cells (*E. coli*), we recommend the "Hanahan protocol" [Hanahan, D., *J. Mol. Biol.* 166, 557 (1983).]
 * Sites are present in some but not all GST Fusion Vectors; consult map of specific vector of interest.

Important Note Concerning Patents
 The use of some vectors for commercial purposes may be subject to patents which either have been issued to or are pending on behalf of third parties. Our customers may need to obtain licenses from such third parties if vectors are to be used for commercial rather than research purposes. This publication shall not be construed as the infringement of any patent, not extending any license, expressed or implied, nor assuming any liability under any issued or pending patents.

Prokaryotic Gene Fusion Vectors

For main product entries,

- GST Gene Fusion Vectors, [see page 431](#).
- pEZZ18 Protein A Gene Fusion Vector, [see page 450](#).

General Cloning Vectors / Cloning Products / Oligonucleotides for Use as Primers

M13mp18 (+) Strand DNA

- For preparation of single-stranded template DNA required for dideoxy sequencing.

Based on the single-stranded bacteriophage M13, the M13 cloning vectors contain multiple cloning sites within a truncated form of the *E. coli lacZ'* gene encoding the α -complementation peptide. The availability of specially designed sequencing primers which hybridize to either side of the MCS allow sequencing in either direction.

Cloning: DNA inserted into the multiple cloning site disrupts the *lacZ'* gene resulting in white plaques when infected cells are grown on media containing X-gal and induced with IPTG (1-5 mM).

Double-stranded sequencing: Inserts can be sequenced from both directions using the M13 Universal Sequencing Primer and M13 Reverse Sequence Primer.

Single-stranded sequencing: The M13 Universal Sequencing Primer can be used with single-stranded DNA. A protocol for production of single-stranded DNA is provided with the vector.

Host(s): A *lac I^q* host is recommended. Infection requires an F' host, such as *E. coli* JM105 or NM522.

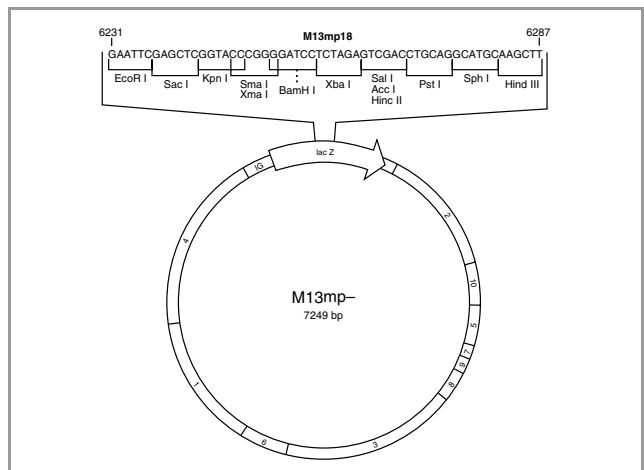
Selectable marker(s): None.

Note: This vector is used as a control for optimizing sequencing reactions. DNA cannot be cloned into this vector.

ORDERING INFORMATION		
Product	Quantity	Code Number
M13mp18 (+) Strand DNA	25 μ g	27-1546-01

For pricing information, visit www.gelifesciences.com/orderonline

GenBank Accession Numbers X02513 (M13mp18 RF).



M13KO7 Helper Phage

For main product entry, see page 450.

ORDERING INFORMATION		
Product	Quantity	Code Number
M13KO7 Helper Phage	100 μ l	27-1524-01

For pricing information, visit www.gelifesciences.com/orderonline

E. coli BL21

For main product entry, see page 451.

Homo-Oligomeric DNA

- For priming DNA synthesis.
- pd(T)₁₂₋₁₈ [also known as oligo(dT)₁₂₋₁₈] is widely used for priming cDNA synthesis.
- Includes both phosphorylated (indicated by "p" preceding symbol for the nucleotide) and nonphosphorylated forms.
- Supplied in lyophilized form.

For information on preparing, storing and determining the concentration of oligonucleotides, see Technical Appendix.

Primers for Sequencing

For main product entries, see page 433.

Polynucleotides

Polynucleotide Nomenclature / Major Polynucleotide Products

Polynucleotide Nomenclature

The following conventions have been adopted to describe our polynucleotides:

- (hyphen) a hyphen between bases symbolizes an alternating repeating base unit on the same strand.
- (dot) a dot placed between two single strands designates two polymers annealed together.
- r the letter (r) indicates a ribonucleotide polymer or oligomer; used to distinguish the ribo-base from the deoxy-base in DNA/RNA hybrids and template/primers.

The table to the right lists the different types of polynucleotides and the nomenclature used to describe them.

TECHNICAL SPECIFICATIONS		
Polynucleotide	Description	Example
Homopolymer	Single-stranded polymer containing only one base	Poly(dA) Poly(C)
Alternating copolymer	Double-stranded polymer containing a 2-base repeating unit designated within parentheses	Poly(dA-dT)·Poly(dA-dT) Poly(rA-rU)·Poly(rA-rU)
Duplex	Double-stranded polymer with complementary strands	Poly(dA)·Poly(dT) Poly(l)·Poly(C)
Template/Primer	Polymer consisting of a sequence-defined oligonucleotide annealed to a single-stranded polynucleotide	Poly(dA)p(dT) ₁₂₋₁₈

4

Cloning & Enzymes

Spectrophotometric quantitation of DNA or RNA

Spectrophotometric measurements of nucleic acid solutions are typically taken at wavelengths of 260 and 280 nm. The A_{260} reading is used to determine the concentration of nucleic acid in solution. For a solution with an $A_{260} = 1.0$, the following approximations hold:

1 A_{260} unit of dsDNA = 50 $\mu\text{g/ml}$

1 A_{260} unit of ssDNA = 37 $\mu\text{g/ml}$ *

1 A_{260} unit of ssRNA = 40 $\mu\text{g/ml}$

* For oligonucleotides, an A_{260} of 1.0 represents anywhere from 20 to 33 $\mu\text{g/ml}$ with the actual conversion factor dependent on the length and base sequence of the oligonucleotide (1).

The ratio between measurements at 260 and 280 nm provides an indication of the purity of a nucleic acid solution. In solution, pure DNA and RNA typically have A_{260}/A_{280} ratios of 1.8 and 2.0, respectively. If the absorbance ratio is significantly less than the values above, the nucleic acid solution is probably contaminated with protein or phenol. Accurate quantitation of a contaminated nucleic acid solution is not feasible without prior purification, and the efficacy of this can be established by the A_{260}/A_{280} ratio.

Most of the lyophilized polynucleotides are sold as A_{260} units (2). For an approximation of quantity, use the conversion factors provided above to convert the A_{260} units into micrograms—which must be known if a certain concentration is desired.

- 1 For a more accurate approximation, refer to Borer in the *Handbook of Biochemistry and Molecular Biology*, 3rd edition (G.D. Fasman, ed.) CRC Press, Cleveland, OH, page 589 (1975).
- 2 Unit definition: One unit is that quantity of oligonucleotide or polynucleotide which has an absorbance of 1.0 at a given wavelength when dissolved in 1 ml of buffer and measured in a 1 cm cuvette at 20°C. The wavelength at which the absorbance is measured is printed on the Certificate of Analysis which accompanies the product. For nucleic acids, typically an absorbance is taken at 260 nm in 20 mM sodium phosphate (pH 7.0), 0.1 M NaCl.

Poly(l)·Poly(C)

- For stimulation of interferon production *in vitro*.
- Supplied as a sterile lyophilized powder.

Since it was discovered that the double-stranded polyribonucleotide poly(l)·poly(C) induces interferon in rabbit cells, there has been much interest in its effectiveness for inducing human interferon *in vitro*.

For extinction coefficients and information on preparing, storing and determining the concentration of polynucleotides, see page 667.

ORDERING INFORMATION		
Product	Quantity	Code Number
Poly(l)·Poly(C)	265 mg	27-4732-01

For pricing information, visit www.gelifesciences.com/orderonline

This product is dispensed by weight of dry material. This weight includes polymer, residual salt and residual water. Content of polymer may vary from lot to lot.

Polynucleotides Listed by Category

For extinction coefficients and information on preparing, storing and determining the concentration of polynucleotides, see page 667.

ORDERING INFORMATION (continued)		
Product	Quantity	Code Number
RNA Homopolymers*		
Poly(rA)	100 mg	27-4110-01
Poly(rA)	500 mg	27-4110-02

For pricing information, visit www.gelifesciences.com/orderonline

*These products are dispensed by weight of dry material. This weight includes polymer, residual salt and residual water. Content of polymer may vary from lot to lot.

Viral DNAs and DNAs for Special Purposes

- **High-quality DNAs from viral and eukaryotic sources used for preparation of assay reagents.**
- Nonmethylated λ DNA (at a concentration of 500 $\mu\text{g}/\text{ml}$)—for preparation of molecular weight markers.
- Methylated λ DNA and nonmethylated λ DNA (at a concentration of 500 $\mu\text{g}/\text{ml}$)—for determining methylation sensitivity of restriction enzymes.
- Activated calf-thymus DNA at 25 A_{260} $\mu\text{g}/\text{ml}$ (treated briefly with DNase I)—for preparing substrate used in the study of DNA polymerases.

ORDERING INFORMATION		
Product	Quantity	Code Number
Viral DNAs		
λ DNA	500 μg	27-4111-01
DNAs for Special Purposes		
DNA (Calf Thymus), Activated	25 A_{260} units	27-4575-01

For pricing information, visit www.gelifesciences.com/orderonline

Sonicated DNAs for Hybridization Studies

- **Lyophilized, sonicated DNAs for reducing hybridization background caused by binding of labeled probe to non-specific sites on membranes.**

ORDERING INFORMATION		
Product	Quantity	Code Number
Sonicated Salmon Sperm DNA, Phenol Extracted	100 A_{260} units	27-4565-01
Sonicated Calf Thymus DNA, Phenol Extracted	100 A_{260} units	27-4563-01

For pricing information, visit www.gelifesciences.com/orderonline

Related Products	Refer To
Amersham Hybridization Oven / Shaker	page 329

MODIFYING ENZYMES GUIDE			
Product group	Refer to	Product group	Refer to
Alkaline Phosphatases	page 140	Polymerases	page 142
Kinases	page 140	Proteases	page 143
Ligases	page 141		
Nucleases	page 141		

Please visit www.gelifesciences.com for a complete list of modifying enzymes available.

Modifying Enzymes

Alkaline Phosphatases / Kinases

Shrimp Alkaline Phosphatase (E.C.3.1.3.1)

- Removes 5'-phosphates from DNA and RNA.
- Easily inactivated by heat.

Source: Arctic shrimp (*Pandalus borealis*).

Description: A high specific activity, heat-labile alkaline phosphatase.

Unit definition: One unit catalyzes the hydrolysis of 1 μ mol of p-nitrophenyl phosphate per minute at pH 10.4 (glycine/NaOH buffer) at 37°C.

Purity: The enzyme is purified to apparent homogeneity and is free of all contaminating endonucleases, exonucleases and ribonucleases.

Activity: 1 unit/ μ l.

Storage conditions: 25 mM Tris-HCl, pH 7.6, 1 mM MgCl₂, 0.1 mM ZnCl₂ and 50% glycerol. Store at -20°C.

ORDERING INFORMATION		
Product	Quantity	Code Number
Shrimp Alkaline Phosphatase	500 units	E70092Y
Shrimp Alkaline Phosphatase	1000 units	E70092Z
Shrimp Alkaline Phosphatase	5000 units	E70092X

For pricing information, visit www.gelifesciences.com/orderonline

Note: Completely and irreversibly inactivated in Tris buffers at pH 8.0–8.5 by heating for 15 min at 65°C. No further treatment is necessary.

Functional testing: Dephosphorylation of a restriction enzyme digested plasmid (5–20 pmol of 5'-ends, 0.1–0.5 units/pmol 5'-ends). Reduces re-ligation to < 0.5% compared to the untreated control.

Supplied with 10 \times reaction buffer: 200 mM Tris-HCl, pH 8.0, 100 mM MgCl₂.

Supplied with dilution buffer: 50 mM Tris-HCl, pH 8.0.

Ready-To-Go T4 Polynucleotide Kinase

- For 5'-end-labeling of DNA or RNA.
- Single-dose, ambient-temperature-stable reaction mixes.

Source: *E. coli* clone.

Description: Catalyzes the transfer of the γ -phosphate of ATP to a 5'-OH terminus in DNA, RNA, 3'-NMP or 3'-dNMP in a process known as the "forward" reaction. The enzyme will also transfer the γ -phosphate to a phosphorylated 5'-terminus through an "exchange" reaction (1). PNK has an associated 3'-phosphatase activity which is part of the same polypeptide (2). Applications include preparation of DNA (3) or RNA (4) for sequencing, and preparation of labeled molecular weight markers and labeled oligonucleotides. Each predispensed, single-reaction, ambient-temperature-stable tube contains a glassified reaction mix which, when reconstituted to a final reaction volume of 50 μ l, yields a solution of reaction buffer, T4 polynucleotide kinase and ATP.

ORDERING INFORMATION		
Product	Quantity	Code Number
Ready-To-Go T4 Polynucleotide Kinase	21 reactions	27-0737-01

For pricing information, visit www.gelifesciences.com/orderonline

Storage conditions: Room temperature.

References

1. Berkner, K.L. and Folk, W.R., *J. Biol. Chem.* **252**, 3176 (1977).
2. Sirotkin, K. et al., *J. Mol. Biol.* **123**, 221 (1978).
3. Maxam, A.M. and Gilbert, W., *Methods Enzymol.* **65**, 499 (1980).
4. Chaconas, G. and Van de Sande, J.H., *Methods Enzymol.* **65**, 75 (1980).

Ready-To-Go T4 DNA Ligase

- For covalently-linking DNA fragments in as little as 30 minutes.
- Single-dose, ambient-temperature-stable reaction mixes.

Description: Catalyzes the formation of a phosphodiester bond between the 5'-phosphoryl group and the 3'-hydroxyl group of two double-stranded DNA fragments. ATP is required for this reaction. Ligation of blunt-ended DNA is greatly stimulated by polyethylene glycol (1). Applications include self-circularization of linear DNA prior to transformation, ligation of insert to vector and ligation of synthetic linkers to blunt-ended DNA (2). Each predispensed, single-reaction, ambient-temperature-stable tube contains a glassified reaction mix which, when reconstituted to a final reaction volume of 20 µl, yields a solution of reaction buffer, T4 DNA ligase, and ATP.

Transformation: A minimum of 1×10^5 colony-forming units per µg of ligated DNA must be obtained when one tube of Ready-To-Go T4 DNA Ligase is reconstituted with 20 µl of a solution containing 100 ng of pUC18/EcoRI/BAP and 100 ng of Kan GenBlock and incubated at 16°C for 30 min.

ORDERING INFORMATION

Product	Quantity	Code Number
Ready-To-Go T4 DNA Ligase	50 reactions	27-0361-01

For pricing information, visit www.gelifesciences.com/orderonline

Blunt-end ligation: > 90% of SmaI fragments of Adenovirus 2 DNA are ligated within 45 min when 1 µg of DNA fragments in 20 µl is incubated with Ready-To-Go T4 DNA Ligase at 16°C.

Cohesive-end ligation: > 90% of HindIII fragments of λ DNA are ligated within 30 min when 1 µg of DNA fragments in 20 µl is incubated with Ready-To-Go T4 DNA Ligase at 16°C.

Storage conditions: Room temperature.

References

1. Pfeiffer, B.H and Zimmerman, S.B., *Nucl. Acids Res.* **11**, 7853 (1983).
2. Helfman, D.M. *et al.*, *Methods Enzymol.* **152**, 349 (1987).

Exonuclease I

- Elimination of residual single-stranded DNA containing a 3'-terminus.
- Measuring endonucleolytic cleavage of covalently closed circular (ccc) ssDNA.
- Measuring DNA helicase activity.

Source: *E. coli* strain containing an overproducing clone of *E. coli* Exonuclease I.

Description: Acts specifically on single-stranded DNA degrading it processively in the 3'- to 5'-direction producing 5'-mononucleotides.

Unit definition: One unit catalyzes the release of 10 nmol of acid-soluble nucleotide from denatured DNA in 30 min at 37°C under standard conditions.

ORDERING INFORMATION

Product	Quantity	Code Number
Exonuclease I	2500 units	E70073Z
Exonuclease I	5000 units	E70073X

For pricing information, visit www.gelifesciences.com/orderonline

Purity: Free of contaminating endonucleases, double-stranded exonucleases and ribonucleases. Greater than 95% pure as determined by SDS-PAGE.

Activity: 10 units/µl.

Storage conditions: 20 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 5 mM 2-mercaptoethanol, and 50% glycerol. Store at -20°C.

Polymerases

Sequenase Version 2.0 T7 DNA Polymerase*

- **DNA sequencing.**

Source: *E. coli* clones that overproduce T7 gene 5 protein and *E. coli* thioredoxin.

Description: Sequenase Version 2.0 T7 DNA Polymerase is a genetically engineered form of T7 DNA polymerase. It has no 3'→5'-exonuclease activity as compared to native T7 DNA polymerase that has a high level of exonuclease activity and Sequenase version 1.0 that has a low but detectable level of exonuclease activity. Like Sequenase Version 1.0, Sequenase Version 2.0 is highly processive, polymerizes DNA rapidly, incorporates useful nucleotide analogues (α -thio dNTPs, ddNTPs, dITP, etc). It is not impeded by secondary structures and will carry out potent strand-displacement synthesis.

Unit definition: One unit catalyzes the incorporation of 1 nmol of nucleotide into acid-insoluble form in 30 s at 37°C.

Activity: 13 units/ μ l.

Assay conditions: The reaction mixture (100 μ l) contains 40 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 5 mM DTT, 0.3 mM dNTPs, and 5 μ g M13mp18 preannealed to 5 pmol M13 universal primer and enzyme. The enzyme is added to the prewarmed (37°C) reaction mixture, incubation is at 37°C for 1 min.

ORDERING INFORMATION		
Product	Quantity	Code Number
Sequenase Version 2.0 T7 DNA Polymerase	200 units	E70775Y
Sequenase Version 2.0 T7 DNA Polymerase	1000 units	E70775Z

For pricing information, visit www.gelifesciences.com/orderonline

Storage: Solution in 20 mM potassium phosphate, pH 7.4, 1 mM DTT, 0.1 mM EDTA, and 50% glycerol. Store at -20°C.

Functional testing: DNA sequencing according to the Sequenase Version 2.0 DNA Sequencing Kit Protocol.

Supplied with 5 \times reaction buffer: 200 mM Tris-HCl, pH 7.5, 100 mM MgCl₂, 250 mM NaCl.

Supplied with dilution buffer: 10 mM Tris-HCl, pH 7.5, 5 mM DTT, 0.1 mM EDTA.

* See licensing information at back of catalog.

References

1. Tabor, S. and Richardson, C. C., *J. Biol. Chem.* **264**, 6447–6458 (1989).
2. Paris, M., *Comments* **18** (No. 3), United States Biochemical Corporation, Cleveland (1992).
3. Tabor, S. and Richardson, C. C., *Proc. Natl. Acad. Sci. USA* **84**, 4767–4771 (1987).
4. Tabor, S. and Richardson, C. C., *Proc. Natl. Acad. Sci. USA* **86**, 4076–4080 (1987).

Thermo Sequenase DNA Polymerase*

(with *Thermoplasma acidophilum* Inorganic Pyrophosphatase (TAP))

- **DNA Sequencing.**
- Genotyping.

Source: *E. coli* clones that overproduce the protein.

Description: Thermo Sequenase is a novel thermostable DNA polymerase that uses dideoxynucleotide triphosphates (ddNTPs) as readily as deoxynucleotide (dNTP) substrates. This results in extremely uniform and easy-to-read sequence band patterns. This property, in addition to thermostability, makes Thermo Sequenase the ideal choice for cycle sequencing where template quantity limitations preclude the use of T7 Sequenase DNA polymerase. The properties of Thermo Sequenase also enable its use in many SNP genotyping applications where primer extension is employed.

Unit definition: One unit of enzyme incorporates 10 nmol of dNTPs into acid-insoluble material in 30 min at 74°C.

Activity: 32 units/ μ l.

Functional testing: Fluorescent 4-dye primers used to sequence single- or double-stranded DNA templates. Accuracy of 98.5% up to 500 base pairs is obtained using an Applied Biosystems model 373 stretch DNA Sequencer using a 34 cm well-to-read distance.

ORDERING INFORMATION		
Product	Quantity	Code Number
Thermo Sequenase DNA Polymerase (with <i>Thermoplasma acidophilum</i> Inorganic Pyrophosphatase (TAP))	1000 units	E79000Y
Thermo Sequenase DNA Polymerase (with <i>Thermoplasma acidophilum</i> Inorganic Pyrophosphatase (TAP))	10000 units	E79000Z

For pricing information, visit www.gelifesciences.com/orderonline

Assay conditions: The reaction mixture (45 μ l) contains 25 mM TAPS buffer, pH 9.3 (at 25°C), 50 mM KCl, 2 mM MgCl₂, 1 mM 2-mercaptoethanol, 200 μ M dATP, dGTP, dTTP, 100 μ M [α -³²P]-dCTP (0.05-0.1 Ci/mmol), and 400 μ g/ml activated DNA. After incubation at 74°C for 10 min, acid-insoluble material is determined.

Storage: Solution in 20 mM Tris-HCl, pH 8.5, 0.1 mM EDTA, 0.5% Tween 20 (v/v), 0.5% Nonidet P-40 (v/v), 1 mM DTT, 100 mM KCl, 50% glycerol. Store at -20°C.

* See licensing information at back of catalog.

Factor Xa

For main product entry, [see page 444](#).

PreScission Protease

For main product entry, [see page 445](#).

Thrombin

For main product entry, [see page 444](#).