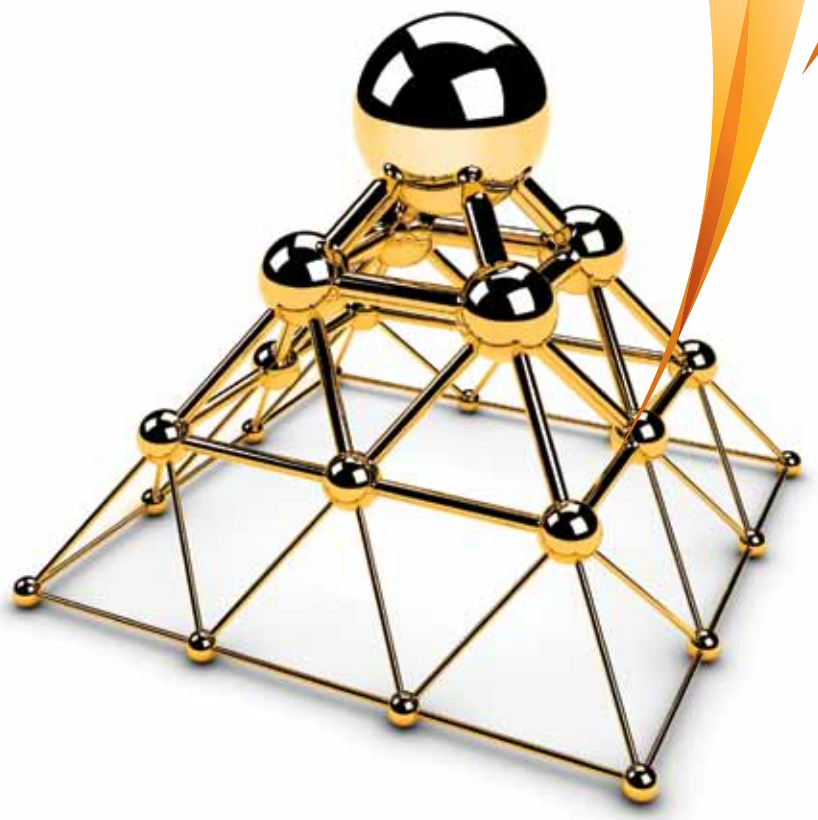
MERCK MILLIPORE

High fidelity gene amplification

PCR tools built for efficient and accurate gene discovery.



Merck Millipore is a division of MERCK

Rise above the competition with Novagen®
PCR Enzymes and Kits.

Successful polymerase chain reaction (PCR) is crucial for amplifying DNA sequences in order to study their function, either by sequencing, mutation, transcription, or expression of gene products.

Having incorporated the expertise of Novagen, Merck Millipore is your source for tools in every step of the gene discovery workflow, including high quality PCR enzymes and reagents, easy-to-use kits, and much needed competent cell platforms to overcome specific challenges in DNA propagation and protein expression. Partner with leading scientists on our technical support and research teams, and watch your research flourish.

For more information

To view and download product-specific information and protocols, visit **www.merckbiosciences.com** and search by product catalogue number.

AccepTor™ Vectors
Covered under US Patent 5,856,144.

KOD Hot Start DNA Polymerase, KOD XL DNA Polymerase, KOD Xtreme™ Hot Start DNA Polymerase, NovaTaq™ DNA Polymerase, NovaTaq™ Hot Start DNA Polymerase, One Step RT-PCR Kit
Use of these products is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224; 5,618,711; 6,127,155 and claims outside the US corresponding to expired US Patent 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California, 94404, USA. KOD DNA Polymerases are manufactured by Toyobo and distributed by Merck Millipore Corp. KOD XL DNA Polymerase is licensed under US Patent No. 5,436,149 owned by Takara Shuzo, Co., Ltd.

OrientExpress™ Systems
Covered under US Patent 5,629,179.

Pellet Paint® Co-Precipitant
Covered under US Patent 7,550,447; 7,144,713, European Patent 0,853,680, and Canadian Patent 2,231,501.

NovaQUANT™ Quantitative PCR (qPCR) Assays
Patent pending.

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01 Amplify DNA Efficiently and Accurately

Thermostable DNA Polymerases

PCR involves replication of the DNA template by a thermostable DNA polymerase. The processivity, specificity, and fidelity of the polymerase enzyme used can influence the efficiency, reproducibility, and yield of the PCR reaction.

Merck Millipore's molecular biologists work to develop and formulate polymerases offering the highest specificity, fidelity and yield during PCR amplification. In addition, optimized buffer compositions, convenient master mixes and cycling parameters provide additional ease of use and data reproducibility. From our premium quality NovaTaq™ DNA polymerase to our ultra-high fidelity KOD polymerases (from *Thermococcus kodakaraensis*), our polymerases save you valuable time and resources.

Our commitment to moving your research forward includes providing products that respect intellectual property law. All Novagen® polymerases are licensed for PCR for research use.

Choosing the appropriate PCR enzyme: qualities to consider

Property of polymerase enzyme:	Importance for PCR:
Elongation Rate (measured in bases incorporated per second)	• Speed of DNA extension • Affects yield of DNA • Shorter PCR cycling (reduced PCR extension rates with faster elongation rates)
Processivity (average number of nucleotides added per association/ disassociation with the template)	Influences overall speed and yield of PCR reaction
Specificity	• Minimizes non-specific amplification of DNA as a result of primer dimerization or mispriming. • Chemical modifications or antibody-mediated methods to block polymerase activity at room temperature also increase amplification specificity compared to standard PCR reaction optimization. (These enzymes are only activated upon heating and therefore are termed "Hot Start" enzymes as listed in our offerings.)
Fidelity	• Fidelity is critical when accurate sequence amplification of the gene target is needed. • Proofreading enzymes (like KOD DNA polymerases) have ultra high fidelity to ensure results are not affected by mutations attributable to PCR amplification.
Challenging target amplification	• Processing crude samples prior to PCR can be time consuming and costly. Merck Millipore's optimized PCR enzyme buffers and ultra high performance KOD enzymes will improve your data turnaround. • The KOD DNA polymerases are also validated for amplification of challenging targets such as GC-rich templates, repeat TA sequences and for long targets or complex PCR needs.

PCR Enzyme Selection Guide

Enzyme	PCR Product Size (kb)	Elongation Rate (bases/s)	Specificity	Fidelity	GC-rich Templates	Yield	PCR Product Ends	Success With Difficult Targets	Applications	Available as Master Mix	Page
KOD DNA Polymerase	<6	120	■	■	■	■	blunt	■	Cloning, cDNA amplification		12
KOD Hot Start DNA Polymerase	<21	120	■	■	■	■	blunt	■	Cloning, cDNA amplification	Y	6
KOD XL DNA Polymerase	<30	120	■	■	■	■	mixed (blunt and 3'-dA)	■	Crude samples, multi-plex, incorporation of derivatized dNTPs		11
KOD Xtreme™ Hot Start DNA Polymerase	<40	120	■	■	■	■	blunt	■	Crude samples, Long targets, difficult and GC-rich targets		9
NovaTaq™ DNA Polymerase	<5	60	■	■	■	■	3'-dA	■	Routine PCR	Y	14
NovaTaq™ Hot Start DNA Polymerase	<5	60	■	■	■	■	3'-dA	■	Hot start routine PCR	Y	13
NovaTaq™ DNA Polymerase plus Taq Antibody	<5	60	■	■	■	■	3'-dA	■	Hot start routine PCR		14

■ Satisfactory ■ Good ■ Excellent ■ Best

KOD polymerases have among the highest fidelity of DNA polymerases.

Fidelity is critical when accurate sequence amplification of the gene target is needed, for example, when direct sequencing or cloning for downstream protein expression. Unwarranted mutation could severely impact your studies. Our analysis has shown that KOD enzymes are an easy choice for fast, accurate and high-yielding PCR.

Enzyme	KOD DNA Polymerase	Pfu DNA Polymerase	Taq DNA Polymerase
Species	*Thermococcus kodakaraensis*	*Pyrococcus furiosus*	*Thermus aquaticus* YT-1
Fidelity† (mutation frequency)	0.0035	0.0039	0.013
Elongation rate (bases/second)	106-138	25	61
Processivity (nucleotide bases)	>300	<20	not determined

† Fidelity was measured by the authors as mutation frequency in PCR products using a sensitive blue/white phenotypic assay with a 5.2-kb lacZ plasmid as template (Takagi 1997).

KOD DNA Polymerase

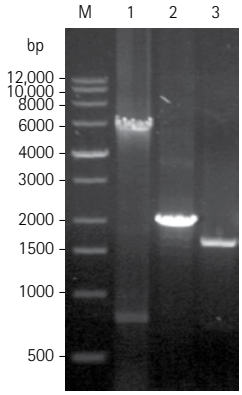
Pure recombinant high fidelity DNA polymerase from *Thermococcus kodakaraensis* KOD1

Features

- High fidelity DNA polymerase: excellent for cloning
- Greater yield: extension speed is 2X faster than Taq DNA polymerase and 5X faster than Pfu DNA polymerase
- Higher processivity: sequential nucleotide polymerization is 10- to 15-fold greater than Pfu and *Tli* DNA Polymerases
- Works well with challenging PCR samples and templates

KOD DNA Polymerase is a recombinant form of *Thermococcus kodakaraensis* KOD1 DNA polymerase[1]. KOD is a high-fidelity thermostable polymerase that amplifies target DNA up to 6 kb with superior accuracy and yield[2]. The 3´→5´ exonuclease-dependent proofreading activity of the enzyme results in a lower mutation frequency even when compared to other high fidelity proofreading polymerase enzymes. Furthermore, KOD DNA polymerase has superior elongation and processivity rates resulting in a highly efficient DNA polymerase. The outcome is highly accurate, fast, and high yielding PCR amplification in the shortest times as compared to conventional enzymes.

1. Nishioka, M. et al. J. Biotechnol. 2001; 88:141.
2. Takagi, M. et al. Appl. Environ. Microbiol. 1997; 63:4504.



Lane	Sample
M	Perfect DNA™ Markers, 0.5–12 kbp
1	5.4-kb PCR product (lambda DNA)
2	2.0-kb PCR product (plasmid DNA)
3	1.6-kb PCR product (human genomic DNA)

PCR products amplified using KOD DNA Polymerase

DNA fragments from various templates were amplified using 2.5 U KOD DNA Polymerase in a 100-µL reaction. Samples were analyzed by agarose gel electrophoresis (1.2% TAE).

Description	Size	Catalogue No.
KOD DNA Polymerase	200	71085-3
Components:		
250 U KOD DNA Polymerase (2.5 U/mL) 1 mL 10X Buffer #1 for KOD DNA Polymerase (pH 8.0) 1 mL 10X Buffer #2 for KOD DNA Polymerase (pH 8.8) 1 mL 25 mM $MgCl^2$ 1 mL dNTP Mix (2 mM each)		

1U is defined as the amount of enzyme that will catalyze the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 min at 75 °C, in a reaction containing 20 mM Tris-HCl (pH 7.5 at 25 °C), 8 mM $MgCl_2$, 7.5 mM DTT, 50 mg/mL BSA, 150 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [3H]dTTP) and150 µg/mL activated calf thymus DNA.

KOD Hot Start DNA Polymerase

Heat-activated KOD DNA Polymerase for increased specificity and convenient PCR setup.

Features

- High accuracy, yield, and processivity compared to most proofreading DNA polymerases
- Amplifies genomic DNA templates up to 12 kb; plasmid and lambda DNA templates up to 21 kb
- Eliminates mispriming and primer-dimer formation
- Inhibition of exonuclease activity at room temperature reduces primer degradation.
- Convenient ambient-temperature setup compatible with automation
- Optimal KOD Hot Start Buffer for robust PCR performance with a wide range of targets
- Compatible with site-directed mutagenesis protocols

KOD Hot Start DNA Polymerase is a premixed complex of high-fidelity KOD DNA Polymerase and two monoclonal antibodies that inhibit the DNA polymerase and 3′→5′exonuclease activities at ambient temperatures[1].

1. Mizuguchi, H et al. J Biochem (Tokyo). 1999; 126:762.

KOD Hot Start DNA Polymerase combines high fidelity with fast, high-yielding DNA amplification compared to polymerases from other suppliers.

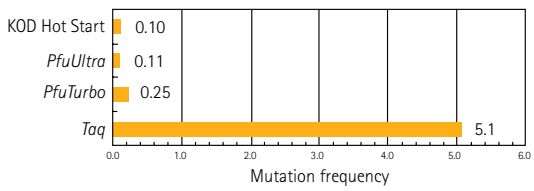
	Cycle Profile A	Cycle Profile B	Cycle Profile C	Cycle Profile D
Initial denaturation	98°C 30 s	94°C 2 min	95°C 2 min	95°C 2 min
29 cycles	98°C 10 s 55°C 20 s 72°C 30 s	94°C 15 s 52°C 20 s 68°C 60 s	95°C 20 s 55°C 20 s 72°C 30 s	95°C 20 s 55°C 10 s 70°C 15 s
Final extension	72°C 5 min	68°C 5 min	72°C 3 min	N/A

Ultra High Fidelity with KOD Hot Start DNA Polymerase

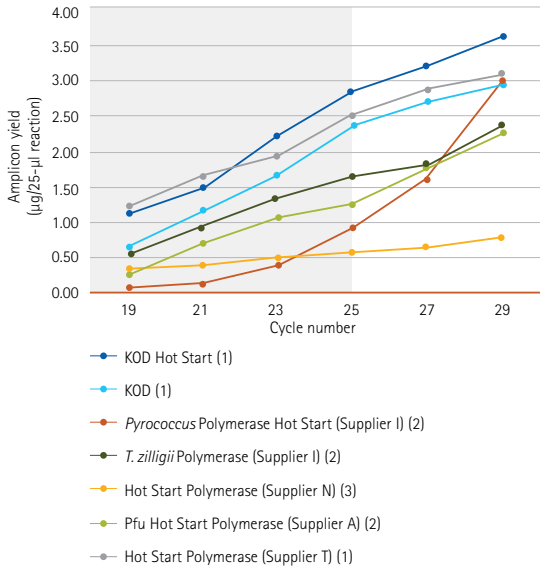
DNA Polymerase	Number of Colonies	
	Total	Mutant
KOD Hot Start	51200	51
Enhanced Pfu Polymerase (Supplier A)	49900	53
Pfu Polymerase (Supplier A)	65900	164
Taq	7000	354
Mutation Frequency: (Number of mutant colonies/Number of total colonies) × 100%		

Percentage of Mutants

Mutation Frequency (%)



The fidelity of replication was measured as the mutation frequency in PCR products using a modified rpsL+ fidelity assay (Kitabayashi 2002, Fujii 1999).



KOD polymerase yields more product in fewer cycles compared to other PCR enzymes. Yields were determined by PicoGreen® analysis after 19, 21, 23, 25, 27, and 29 cycles for all 4 cycling profiles. The best yield data for each enzyme, from any cycling profile, were graphed. The cycling profile that gave the best yield is identified in parentheses. The shaded area highlights yields in cycles 19-25, which are most preferred for cloning. KOD-HS DNA polymerase outperformed the competition.

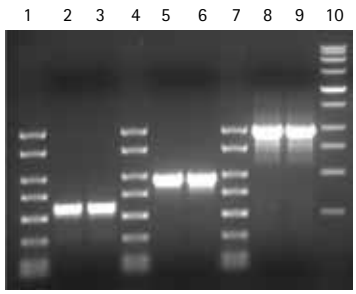
Description	Size	Catalogue No.
KOD Hot Start DNA Polymerase	20 U	71086-5
	200 U	71086-3
	1,000 U	71086-4
Components:		
20 U, 200 U, or 5 x 200 U KOD Hot Start DNA Polymerase (1.0 U/µL) 1.2 mL or 5 x 1.2 mL 10 x PCR Buffer 1 mL or 5 x 1 mL 25 mM $MgSO_4$ 1 mL or 5 x 1 mL dNTP mix (2 mM each)		

KOD Hot Start Master Mix

Speed up your PCR with convenient setup, higher throughput, higher accuracy and reproducibility with fewer steps, preventing contamination.

Features:

- High accuracy, yield and processivity compared to most commercially available proofreading DNA polymerases
- Amplifies 12 kb (genomic) and 21 kb (plasmid or lambda) DNA templates
- Eliminates mispriming and primer-dimer formation
- Convenient ambient-temperature setup with premixed 2x KOD Hot Start PCR components compatible with automation
- Optimal KOD Hot Start Buffer for robust PCR performance with wide range of targets (crude sample, long PCR and high GC/TA content).



Lane	Template – Target - Size
1	PCR Markers
2	λ DNA – *att* region - 595 bp
3	λ DNA – *att* region - 595 bp
4	PCR Markers
5	cDNA plasmid – GSK 3α CD ORF – 919 bp
6	cDNA plasmid – GSK 3α CD ORF – 919 bp
7	PCR Markers
8	Uncut BacVector® 3000 DNA – Chitinase deletion region – 1.9 kb
9	Uncut BacVector® 3000 DNA – Chitinase deletion region – 1.9 kb
10	Perfect DNA™ Markers, 0.5-12 kb

The indicated DNA fragments were amplified using KOD Hot Start Master Mix in separate 50 µL or 20 µL reactions using cycling conditions indicated in the user protocol. Samples (5 µL) were analyzed by agarose gel electrophoresis and stained with ethidium bromide.

Description	Size	Catalogue No.
KOD Hot Start Master Mix	100 rxm	71842-3
	500 rxm	71842-4

KOD Xtreme™ Hot Start DNA Polymerase

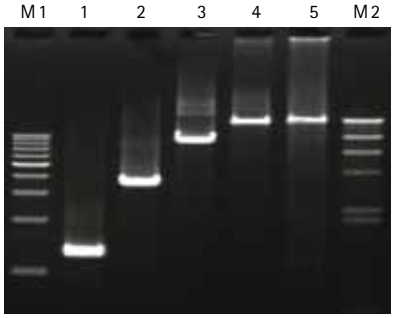
Optimized PCR system for the most challenging samples or DNA templates

Features

- Optimized for PCR success against complex crude samples and with minimal processing
- Efficiently amplifies up to 90% GC-content templates
- 10x higher fidelity than Taq blends
- Amplifies genomic targets up to 24kb
- Amplifies plasmid/phage targets up to 40kp
- Eliminate mispriming or prime-dimer formation
- Convenient ambient-temperature set up compatible with automation

The KOD Xtreme™ Hot Start (HS) DNA Polymerase is your enzyme of choice for the most challenging PCR situations, including crude samples, high GC content, or repeat sequences (T/A) which can inhibit or bias PCR amplification data. This is the most relied-upon enzyme for PCR from blood, from lysates of complex microorganisms, and for minimally processed animal or plant tissue. KOD Xtreme™ HS DNA polymerase and its optimized buffer conditions offer remarkable properties (see enzyme selection guide on page 5) that exceed the capabilities of comparable DNA polymerases.

Efficient and accurate amplification of long genomic DNA targets



Lane Samples

M1	1 kb DNA ladder
1	1.3 kb β-globin target
2	3.6 kb β-globin target
3	8.5 kb β-globin target
4	17.5 kb β-globin target
5	24 kb tissue plasminogen activator target
M2	λ /HindIII DNA Markers

The indicated targets were amplified from 200 ng human genomic DNA. PCR cycling parameters for 1.3 to 8.5 kb targets: 94 °C for 2 min; 30 cycles at 98 °C for 10 s, 68 °C for 1 min/kb. PCR cycling parameters for 17.5 and 25 kb targets: 94 °C for 2 min; 5 cycles at 98 °C for 10 s, 74 °C for 1 min/kb; 5 cycles at 98 °C for 10 s, 72 °C for 1 min/kb; 5 cycles at 98 °C for 10 s, 70 °C for 1 min/kb; 20 cycles at 98 °C for 10 s, 68 °C for 1 min/kb.

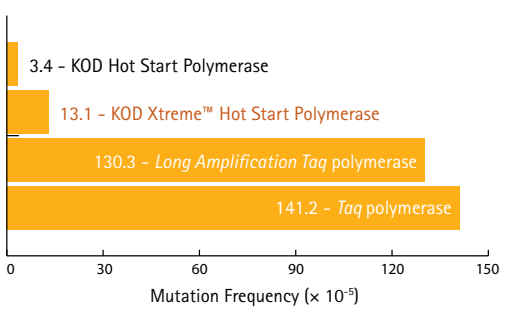
Ultra High Fidelity of KOD Hot Start and KOD Xtreme™ Hot Start DNA Polymerases during long PCR amplification.

DNA Polymerase	Number of Bases Sequenced	Number of Bases Mutated
KOD Hot Start	145,753	5
KOD Xtreme™	144,535	19
Long Amplification Taq (Supplier T)*	167,343	218
Taq	102,708	145

* Long Amplification Taq is representative of polymerases mixes which are a blend of Taq DNA polymerase and a proofreading enzyme

Percentage of Mutants

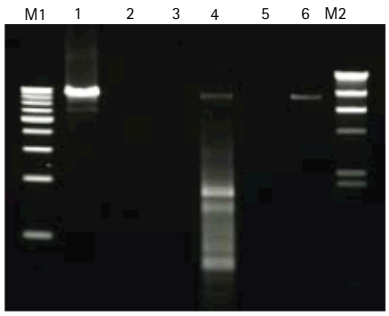
Mutation Frequency (%)



01

Amplify DNA Efficiently and Accurately
Thermostable DNA Polymerases

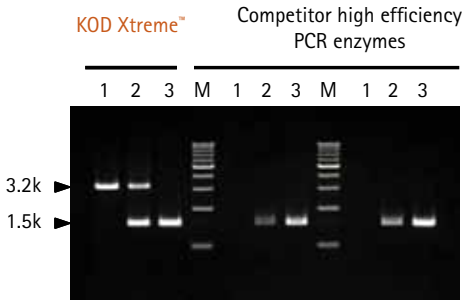
Optimized PCR amplification for GC-rich DNA sequences



Lane Samples
M1 1 kb DNA ladder
1 KOD Xtreme™ Hot Start DNA Polymerase
2 Taq Polymerase (Supplier T)
3 Long Amplification Taq Polymerase (Supplier T)
4 Brand PS Polymerase w/ GC Buffer (Supplier T)
5 Long Amplification Taq Polymerase w/ GC Buffer 1 (Supplier T)
6 Long Amplification Taq Polymerase w/ GC Buffer 2 (Supplier T)
M2 λ.HindIII DNA Markers

KOD Xtreme™ Hot Start Polymerase amplifies GC-rich targets more efficiently than other polymerases. Six polymerases shown were used to amplify a 90% GC-containing region of human IGF2R [NM_000876] from HeLa cell cDNA. PCR cycling parameters for KOD Xtreme polymerase were: initial denaturation at 94 °C for 2 min, 30 cycles at 98 °C for 10 s, and 68 °C for 9 min. For polymerases from other manufacturers, optimal recommended parameters were used.

Amplify DNA from crude tissue lysate with minimal processing to save time and minimize cost while maintaining quality data.



M: 1kb DNA Ladder
1: Tg/Tg
2: Tg/W
3: W/W

The targeted locus genes were amplified with various PCR enzymes using mouse tail lysates prepared by alkaline lysis*. KOD Xtreme™ Hot Start DNA polymerase successfully amplified both targets (Tg and WT).

*Lysis method: 1) Transfer mouse tails (3 mm) to 96-well PCR plate or PCR tubes. 2) Add 180 µL of 50 mM NaOH, vortex, and spin. 3) Incubate at 95 °C for 10 min. 4) Add 20 µL of 1 M Tris-HCl (pH 8.0), vortex, and spin. 5) Use 0.5-2 µL supernatant in 50 µL PCR reactions.

Description	Size	Catalogue No.
KOD Xtreme™ Hot Start DNA Polymerase	200 U	71975-3
Components:		
1 x 200 U Polymerase 3 x 1.7 mL 2x Xtreme™ Buffer 2 x 1 mL dNTPs (2 mM each)		

KOD XL DNA Polymerase

High performance enzyme blend for long and accurate PCR

Features

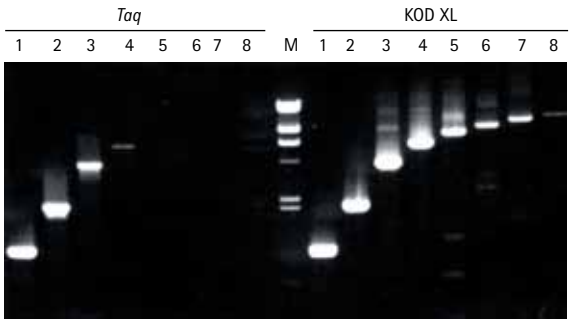
- High-yielding, accurate amplification of large DNA fragments from purified DNA or crude samples
- Amplifies lambda and plasmid DNA up to 30 kb
- Successfully amplifies GC-rich sequences
- Excellent for incorporation of deriviatized dNTPs
- PCR products are a mixture of blunt and sticky ends (3'-dA overhangs)

KOD XL DNA polymerase is an optimized blend of KOD DNA polymerase and a mutant form of KOD that is deficient in 3'-5' exonuclease activity[1]. It can be used for successful incorporation of deriviatized dNTPs in PCR amplicons[2,3]. While other KOD enzyme formulations generate blunt ended PCR products, KOD XL DNA polymerase generates a mixture of PCR products with blunt and 3'-dA overhangs.

References:

1. Nishioka, M et al. 2002 J. Biotechnology 88, 141
2. Sawai, H et al. 2002. Bioconjugate Chem. 13, 309
3. Sawai, H., et al. 2001 Chem. Commun. 24, 2604

KOD XL DNA Polymerase is a high yielding blend formulation for long PCR.



Lane	Sample
1	1-kb PCR fragment
2	2-kb PCR fragment
3	4-kb PCR fragment
4	6-kb PCR fragment
5	8-kb PCR fragment
6	10-kb PCR fragment
7	12-kb PCR fragment
8	15-kb PCR fragment

DNA fragments were amplified using 2.5 U Taq or 2.5 U KOD XL DNA polymerase in 50 µL reactions. M: markers.

Description	Size	Catalogue No.
KOD XL DNA Polymerase	250 U	71087-3
	1,250 U	71087-4
Components:		
250 U or 5 x 250 U KOD XL DNA Polymerase (2.5 U/µL) 1.2 mL or 5x 1.2 mL 10x PCR Buffer for KOD XL DNA Polymerase 1 mL or 5 x 1 mL dNTP mix (2 mM each)		

NovaTaq™ DNA Polymerases: High-yielding, sensitive polymerases for improved efficacy in routine PCR

Ultrapure recombinant enzyme for dependable, routine PCR amplification

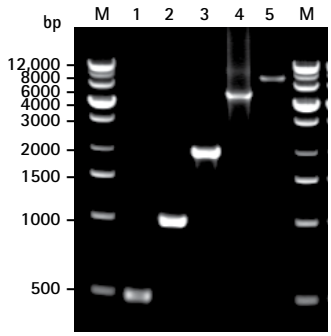
Features of NovaTaq™ DNA Polymerases

- Higher PCR specificity and yield
- Improved low-copy target amplification
- Ambient temperature setup compatible with automation
- Amplifies targets up to 5 kb
- Ideal for quantitative and high throughput PCR applications

NovaTaq™ DNA polymerase

NovaTaq™ DNA polymerase is a premium quality recombinant form of *Thermus aquaticus* (Taq) DNA polymerase. NovaTaq™ DNA polymerases are suitable for wide range of qualitative or quantitative PCR amplification needs. To ensure the highest purity and reproducible performance, each preparation is extensively tested in a variety of control assays. NovaTaq™ DNA polymerase has 5′ -3′ exonuclease activity and lacks 3′ - 5′ exonuclease activity, which results in 3′-dA overhangs.

NovaTaq™ DNA Polymerase generates high yields of pure amplified DNA



Lane	Sample
M	Perfect DNA™ Markers, 0.5–12 kb
1	0.5-kb PCR product
2	1.0-kb PCR product
3	2.0-kb PCR product
4	4.8-kb PCR product
5	7.35-kb PCR product

PCR products amplified using NovaTaq™ DNA Polymerase DNA fragments 0.5 to 7.35 kb in size were amplified using 2.5 U NovaTaq™ DNA Polymerase in separate 100 mL reactions. Products were analyzed by agarose gel electrophoresis (1.2% TAE).

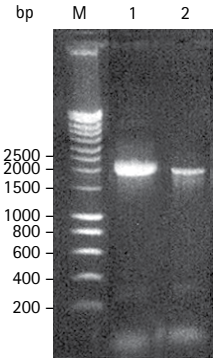
Description	Size	Catalogue No.
NovaTaq™ DNA Polymerase	100 U	71003-3
	500 U	71003-4
	2,500 U	71003-5
Components:		
100 U, 500 U, or 5x 500 U NovaTaq™ DNA polymerase 1 x 1.5 mL, 2x 1.5 mL, or 7 x1.5 mL 10x NovaTaq™ Buffer with $MgCl_2$ 1 x 1.5 mL, 2x 1.5 mL, or 7 x1.5 mL 10x NovaTaq™ Buffer without $MgCl_2$ 1 x 1.5 mL, 2x 1.5 mL, or 7 x1.5 mL $MgCl_2$		

Unit definition: one unit is defined as the amount of enzyme that will catalyze the incorporation of 10 nmol dNTP into an acid-insoluble form in 30 min at 74 °C, in a reaction containing 25 mM TAPS (tris-[hydroxymethyl]-methyl-amino-propane-sulfonic acid, sodium salt), pH 9.3 at 25 °C, 50 mM KCl, 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.2 mM dATP, dGTP, and dTTP, 0.1 µM [α-32P] dCTP, and activated salmon sperm DNA. Additional Information in user protocol TB309

NovaTaq™ Hot Start DNA Polymerase

Heat-activated, chemically modified form of recombinant Taq DNA polymerase

NovaTaq™ Hot Start DNA polymerase offers better PCR yields than other leading commercial Taq polymerases



Lane	Sample
M	Markers
1	2-kb fragment amplified using NovaTaq™ Hot Start DNA Polymerase
2	2-kb fragment amplified using Company A chemically modified *Taq* DNA polymerase

Indicated fragments were amplified under standard hot start PCR conditions for each enzyme and analyzed by agarose gel electrophoresis.

Description	Size	Catalogue No.
NovaTaq™ Hot Start DNA Polymerase	250 U	71091-3
	1,250 U	71091-4
Components:		
250 U or 5 x 250 U NovaTaq™ Hot Start DNA Polymerase 1.5 mL or 5 x 1.5 mL 10x NovaTaq™ Hot Start Buffer 1.5 mL or 5 x 1.5 mL 25 mM $MgCl_2$		

Unit definition: One unit is the amount of enzyme that will catalyze the incorporation of 10 nmol of dNTP into an acid-insoluble form in 30 min at 72˚C, in a reaction containing 25 mM TAPS (tris-[hydroxymethyl]-methyl-amino-propanesulfonic acid, sodium salt), pH 9.3 at 25˚C, 50 mM KCl, 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.2 mM dATP, dGTP, and dTTP, 0.1 µM [α-32P]dCTP, and 12.5 µg activated salmon sperm DNA in a volume of 50 µL. Additional Information in User Protocol TB460

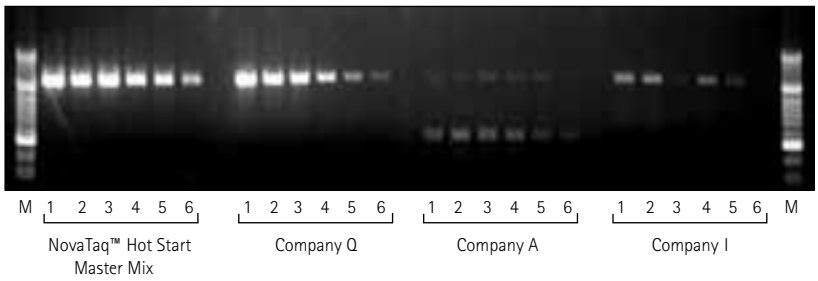
NovaTaq™ Master Mix Kits

Premixed 2X PCR components for convenience and reproducibility

Features

- Ready-to-use, 2x mixture of NovaTaq™ or NovaTaq™ Hot Start DNA polymerase, ultrapure dNTPs and PCR buffer
- All the benefits of NovaTaq™ or NovaTaq™ Hot Start DNA polymerase with simple set up
- Quick and reproducible data turnaround
- Reduced risk for contamination
- Compatible with high throughput PCR
- Separate PCR-grade water and $MgCl_2$ for additional reaction optimization

Sensitive amplification from low target concentrations



M-Markers

Lane	template DNA (pg)
1	125.0
2	41.7
3	13.9
4	4.6
5	1.5
6	0.5

A 1 kb fragment from lambda DNA was amplified using the indicated master mix in a 50 µL reaction. Cycling parameters were as follows: 95 °C for 8 min, 60 °C for 1 min; 30 cycles at 95 °C for 30s, 60 °C for 30 s, 72 °C for 15 s; final extension at 72 °C for 10 min. Samples were analyzed by agarose gel electrophoresis and stained with ethidium bromide.

Description	Size	Catalogue No.
NovaTaq™ PCR Master Mix	200 rxn	71007-3
Components:		
4 x 1.25ml 2 x NovaTaq™ PCR Master Mix 1.5 ml $MgCl_2$ Solution 3 x 2ml PCR Grade Water		
NovaTaq™ Hot Start Master Mix Kit	200 rxn	71676-3
	1,000 rxn	71676-4
Components:		
4 x 1.25 mL or 20 x 1.25 mL NovaTaq™ Hot Start Master Mix 1 x 1.5 mL or 3 x 1.5 mL $MgCl_2$ 3 x 2 mL or 11 x 2 mL PCR-Grade Water		

01

Amplify DNA Efficiently and Accurately

Reverse Transcription and PCR (RT-PCR) Kits

Using reverse transcription (RT) to convert gene transcripts or RNA targets such as messenger RNAs (mRNAs) to complementary DNA (cDNA) is a necessary step for PCR amplification. Gene expression profiling (eg mRNA detection and quantification) between experimental conditions using RT-PCR has become a key step in many cell culture or in-vivo animal model experiments. To simplify this process, Merck Millipore has designed One Step RT-PCR reagents to efficiently and rapidly convert RNA to cDNA for PCR amplification in a single tube. This process minimizes the risk of RNA degradation and contamination during the RT-PCR process in a single reaction for convenient setup and fast data turnaround. Achieve simpler end-point PCR analysis from RNA templates or obtain more comparative, reproducible and accurate gene expression data in quantitative real-time PCR settings.

One Step RT-PCR Master Mix Kit

Convenient one-enzyme, hot start master mix system for RT-PCR

Features

- Robust one-step enzyme master mix system for easy reaction assembly
- Eliminate risk of cross contamination associated with two-step RT-PCR protocols
- High temperature (60 °C) for reverse transcription enhances read-through of RNA secondary structure
- Ideal for gene expression studies from tissues or cells
- Compatible with quantitative (real-time) RT-PCR
- Optimized buffer conditions and antibody-mediated hot start for increased sensitivity and specificity of both RT and PCR.
- Rapid enzyme activation step (30 s) avoids damage of template RNA

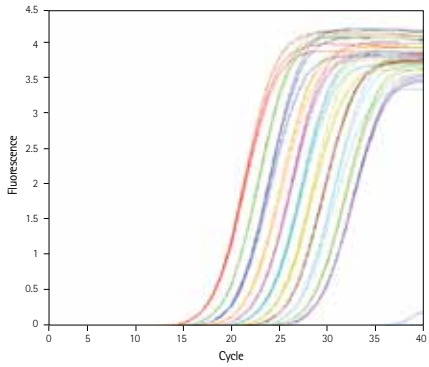
This kit uses recombinant *Thermus thermophilus* (r*Tth*) DNA Polymerase, which acts as both a thermostable RNA-dependent DNA polymerase and a DNA-dependent DNA polymerase. It can eliminate the need for technically demanding Northern blots, *in situ* hybridization, S nuclease assays or conventional two-step RT-PCR. This 2x RT-PCR Master Mix includes antibody-mediated hot start r*Tth* DNA polymerase, optimized buffer and dNTPs. The single input of RNA leading to RT followed by immediate PCR minimizes time to process samples and reduces risk of experimental contamination and cost.

01

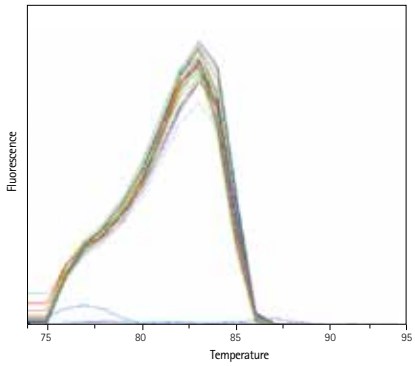
Amplify DNA Efficiently and Accurately

Reverse Transcription and PCR (RT-PCR) Kits

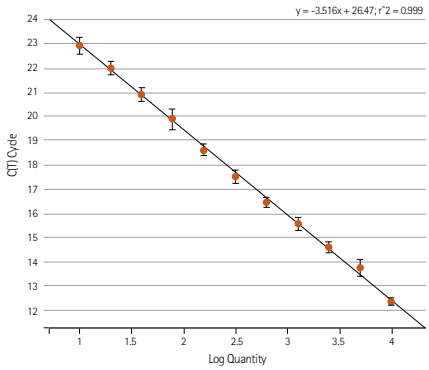
One-step amplification with real-time analysis of Cyclophilin B gene transcripts using the One Step RT-PCR Master Mix Kit and crude cell lysates.



Panel A: Amplification Curve



Panel B: Melting Curve



Panel C: Ct vs. Cell Number

HeLa cells were pelleted and treated with CytoBuster™ Protein Extraction Reagent (Cat No. 71009) and RNAse Inhibitor (Cat. No. 556881). Lysates were two-fold serially diluted in TE + 0.02% Triton X-100. For qPCR, 2 µL of each dilution was used as template in 50 µL RT-PCR reactions done in triplicate. A 99 bp amplicon from cyclophilin B was amplified (Bio-Rad thermocycler and SYBR® Green) using following cycling conditions: 30 s at 90°C (1x), RT for 15 min at 60°C (1x), denaturation for 30 s at 94°C (1x), followed by 40 cycles of denaturation for 1 s at 95°C, annealing for 15 s at 50°C and extension for 5 s at 72°C. Excellent amplicon yield and reproducibility is shown in A. The specificity of the assay is illustrated with a single product amplification as shown by melting curve analysis (B).The signals titrated from 10^4 – 10 cells showed high linearity (correlation coefficient of 0.999 (C)).

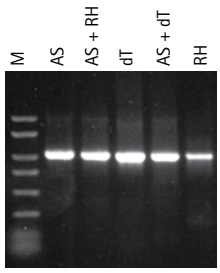
Description	Size	Catalogue No.
One Step RT-PCR Master Mix	50 rxn	71978-3
Components:		
2 × 625 µL 2X One Step RT-PCR Master Mix 1 × 200 µL 50 mM $Mn(OAc)_2$ 1 × 1.1 mL RNase-Free Water 1 × 50 µL Primer F (10 pmol/µL) 1 × 50 µL Primer R (10 pmol/µL) 1 × 50 µL Positive Control RNA (5 × 10^8 copies/µL)		

First Strand cDNA Synthesis Kit

Reliable preparation of templates for RT-PCR

The First Strand cDNA Synthesis Kit is designed for the preparation of high-quality first strand cDNA from RNA templates. The kit contains MMLV Reverse Transcriptase for superior yields of full-length cDNA. Both oligo(dT) and random hexamer primers are included for a choice of general priming strategies and as alternatives to user-supplied specific primers. A small volume (1-2 µL) of the first strand cDNA reaction can be used in PCR amplification with KOD DNA Polymerase. Use this kit with the Straight A's™ mRNA Isolation System (Catalogue No. 69963) and appropriate PCR reagents to amplify rare coding regions.

Efficient generation of first strand cDNA



Positive control RNA (included in kit) was subjected to first strand cDNA synthesis with various primer combinations followed by addition of the 5'sense Control Primer (and antisense Control Primer, where appropriate) and amplification. First strand primers are indicated. AS: antisense Control Primer; RH: random hexamers; dT: oligo(dT).

Description	Size	Catalogue No.
First Strand cDNA Synthesis Kit	40 rxn	69001-3
Components:		
4000 U MMLV Reverse Transcriptase 200 µL 5X First Strand Buffer 100 µL 100 mM DTT 50 µL 10 mM dNTP Mix 20 µg Oligo(dT) Primer 10 µg Random Hexamer Primers 1.5 µL Nuclease-free Water 100 pmol Positive Control Primer, 3' AS 100 pmol Positive Control Primer, 5' S		
Oligo (dT) primer	20 µg	69896-3

01

Amplify DNA Efficiently and Accurately

Reverse Transcription and PCR (RT-PCR) Kits

OrientExpress™ cDNA Synthesis and Cloning Kits

Rapid and efficient construction of cDNA libraries having inserts in a defined orientation for vector cloning

Features:

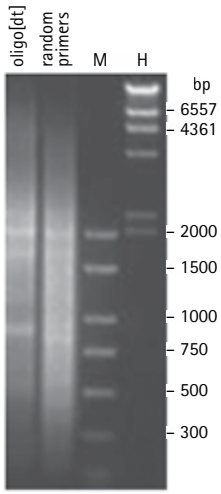
- Complete set of reagents for rapid, efficient construction of cDNA libraries
- Defined orientation of cDNA library inserts
- Libraries cloned into Bacteriophage T7 Vectors (T7Select® 1-1 and T7Select® 10-3)
- Kit designs for random or oligo(dT) priming
- Large library production

The OrientExpress™ cDNA synthesis and cloning systems use either a patented, directional random priming strategy or utilize a orientation-specific oligo(dT) priming to clone between EcoR1 and Hind III sites. Starting with high-quality poly(A)+ RNA, five libraries can be produced with either system.

OrientExpress™ System Vectors

Vector	Applications	Features
T7Select® 1-1	T7 Phage libraries High-affinity selection Display of large proteins Large libraries	Biopanning 0.1-1 copy target/phage Up to 3.6 kb (1200aa) capacity Ultra-high cloning efficiency
T7Select® 10-3	T7 Phage Libraries Medium-high affinity selection Plaque lift detection Large libraries	Biopanning 5-15 copies of target/phage Easy verification of ligand binding Up to 3.6 kb (1200aa) capacity Ultra-high cloning efficiency

Successful, directional cDNA synthesis



Poly(A)+ RNA purified from rat liver using the Straight A's™ System was used for cDNA synthesis with the OrientExpress™ oligo(dT) and random primers as indicated. The cDNA products were analyzed by agarose gel electrophoresis. Lambda HindIII markers (H) and Novagen® PCR Markers (M) were run as size standards.

Description	Size	Catalogue No.
OrientExpress™ Random Primer cDNA Synthesis Kit	1 kit	69993-3
OrientExpress™ Oligo(dT) cDNA Synthesis Kit	1 kit	69992-3
Components:		
5 µg Hind III Random Primers (or 10 µg Oligo(dT)) primer 4000 U MMLV Reverse Transcriptase 50 µL First Strand Buffer 250 U DNA Polymerase I 8 U RNAse H 250 µL Second Strand Buffer 50 µL 10 x Methylation dNTP Mix 100 µL 100 mM DTT 1.5 mL Nuclease-free water 250 µL 10 mg/mL Glycogen 1.8 mL 4 M Ammonium Acetate 5 µg OrientExpress™ Positive Control RNA		
T7Select®1-1 OrientExpress™ cDNA Cloning System, Oligo (dT)	1 kit	70200-3
T7Select®1-1 OrientExpress™ cDNA Cloning System, Random Primer	1 kit	70202-3
T7Select®10-3 OrientExpress™ cDNA Cloning System, Random Primer	1 kit	70580-3
T7Select®10-3 OrientExpress™ cDNA Cloning System, Oligo(dT)	1 kit	70581-3
Components:		
1 OrientExpress™ Primer; Oligo(dT) random primer Synthesis Kit 1 EcoRI/HindII End Modification Kit 1 Mini Column Fractionation Kit 1 DNA Ligation Kit 1 T7Select®1-1 or 10-3 Cloning Kit		
Additional Information:		
TB247 OrientExpress™ cDNA Manual TB178 T7Select® System Manual		

01

Amplify DNA Efficiently and Accurately
NovaQUANT™ Quantitative PCR (qPCR) Assays

Detect genes of interest with high specificity and sensitivity with optimized primer pairs using qPCR. We've done all the work for you, providing validated, pre-aliquoted primers and ready-to-use protocols so you can focus on biology instead of assay development.

NovaQUANT™ Human and Mouse Mitochondrial to Nuclear DNA Ratio Assays

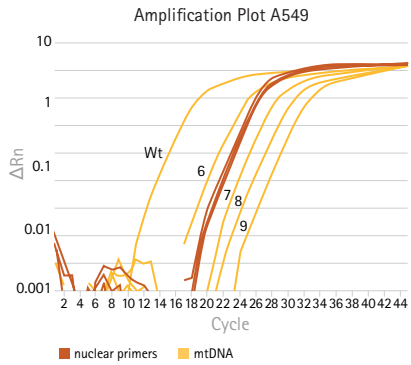
Simple, accurate, real-time detection of mitochondrial DNA.

Features

- Optimized mitochondrial & nuclear primer pairs for matched amplification efficiencies
- Validated gene targets to eliminate mitochondrial pseudogene bias
- Optimized protocol for sensitive and linear quantification
- Specificity control DNA (wild type and mitochondrial negative) total DNA
- Convenient platform with pre-aliquoted primers

Mitochondrial research is an expanding field with few standardized experimental tools. NovaQUANT™ qPCR assays are an innovative, reliable and user-friendly way to determine the ratio of mitochondrial to nuclear DNA. This ratio is one measure of mitochondrial content in the cell. The accurate determination of this ratio is key to assessing cellular homeostasis, which can change with respect to cell differentiation, stress, disease, exercise, caloric intake, and toxicity. As a result, NovaQUANT™ assays can play a pivotal role in almost every area of biological research, including toxicology, metabolic disease, and cancer.

Sensitive and linear detection of key mitochondrial and host cell genes using SYBR® Green Technology



Ethidium Bromide (EtBr)-treated A549 Human Lung Epithelial Carcinoma Cells were cultured in 50 ng/mL EtBr with passage numbers indicated in black next to curves. EtBr is concentrated differently in the mitochondria due to higher mitochondrial membrane potential and subsequent DNA binding. Cells were directly lysed in PCR reactions, total DNA normalized to 1 ng/mL and tragets amplified using paired mitochindral (mtDNA) or nuclear primers in a NovaQUANT™ qPCR assay with SYBR® Green Technology. Higher passage numbers lead to greater depletion of mtDNA as cells transition to a glycolytic energy state. Dark lines show no change in the nuclear DNA. Wt equals wildtype.

Description	Size	Catalogue No.
NovaQUANT™ Human Mitochondrial to Nuclear DNA Ratio Assay	1 kit	72620-1KIT
NovaQUANT™ Human Mitochondrial to Nuclear DNA Ratio Assay	1 kit	72621-1KIT
Components:		
Two qPCR plates with pre-aliquoted PCR primer pairs Wild type Total DNA (100 ng) Mitochondrial Minus Rho Zero DNA (100 ng)		

NovaQUANT™ Human or Mouse Mitochondrial Oxidative Stress and Biogenesis qPCR Assays

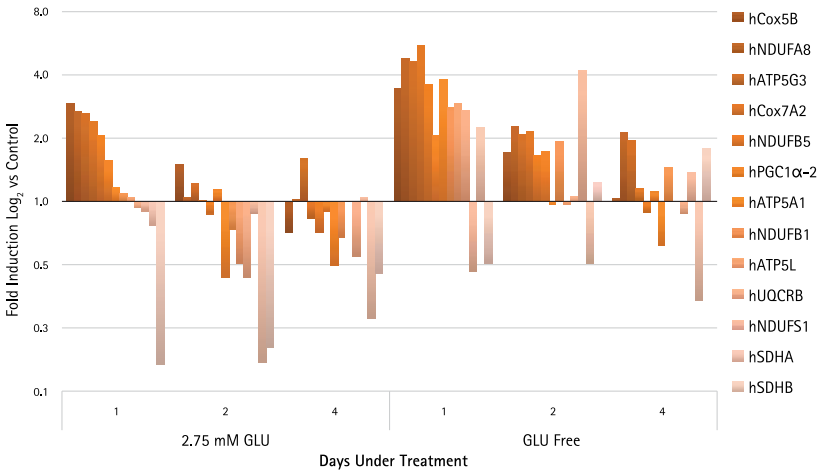
Simple, accurate, real-time profiling of mitochondrial gene expression

Features

- Validated primer sets ensure accurate quantification
- Verified gene targets from in-house and field publications for both human and mouse samples eliminate time-consuming target validation
- Highly specific panel of 20 transcriptionally regulated oxidative stress genes for unambiguous interpretation
- Panel of 12 biogenesis gene targets tightly controlled by master regulator gene PGC1A
- Stable housekeeping genes offering suitable normalization controls across cell types and experimental conditions for improved reproducibility
- Pre-aliquoted plate formats with high quality cDNA controls for ultimate convenience

Primary research into cell health or toxicity research, requires a targeted approach to quantify key mechanisms behind changes in mitochondrial and cellular conditions. NovaQUANT™ mitochondrial gene panels enable qPCR expression profiling of key transcriptionally regulated genes associated with mitochondrial biogenesis and oxidative stress, enhancing studies of metabolism, cell stress, cardiovascular disease, neurodegeneration, toxicity, cell differentiation, immunology, and much more.

Glucose Deprivation Affects Transcription of Mitochondrial Electron Transport Chain Subunit Genes As Detecting Using the NovaQUANT™ Human Mitochondrial Biogenesis Panel



To study the effects of glucose starvation on gene expression in the Electron Transport Chain complexes, HepG2 Cells were cultured in full-glucose (5.5 mM), low-glucose (2.75 mM) or glucose-free Medium. RNA was isolated at days 1, 2 and 4 post-treatment and expression profiling performed using the NovaQUANT™ human mitochondrial biogenesis panel with One Step RNA-direct SYBR® Green Real-time PCR master mix (Toyobo) using a StepOnePlus Real Time PCR Instrument (Life Tech ŁtApplied Biosystems). Following normalization to control group (5.5 mM glucose), a marked response in gene expression was shown 24 hours in both low glucose and glucose free samples respectively. Subunits from Complex I, IV and V were upregulated in the glucose-free and low-glucose samples at 24 hours. Interestingly, both subunits of Complex II (Succinate Dehydrogenase) were down-regulated over all three time points in the low-glucose treatment group. Complex II links Oxidative Phosphorylation to the Krebs cycle and glucose utilization; therefore, this observation is consistent with a lower glucose level leading to decreased Complex II utilization.

Description	Size	Catalogue No.
NovaQUANT™ Human Mitochondrial Biogenesis qPCR Kit	1 kit	72625-1KIT
NovaQUANT™ Mouse Mitochondrial Biogenesis qPCR Kit	1 kit	72626-1KIT
NovaQUANT™ Human Mitochondrial Oxidative Stress qPCR Kit	1 kit	72627-1KIT
NovaQUANT™ Mouse Mitochondrial Oxidative Stress qPCR Kit	1 kit	72628-1KIT
Components:		
Two qPCR plates* with pre-aliquoted primer panel HepG2 cDNA or mouse fibroblast LM cDNA (both 100 ng at 10 ng/µL)		

* Primers are plated into Applied Biosystems MicroAmp® Fast Optical 96-well Reaction Plate with barcode, 0.1 mL (Cat. No. 4346906), which is compatible with 7500 Fast Real-Time PCR system, 7900HT FAST Real-Time PCR System, and StepOnePlus™ Real-Time PCR Systems.

02 Meet Technical Demands in PCR Clean-Up and Nucleic Acid Preparation

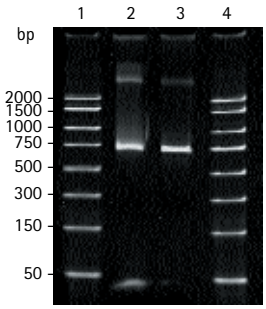
Centrifugal Technologies

Meeting increasingly stringent technical demands in preparing samples of amplified DNA requires reliable tools manufactured with strict quality control. Downstream manipulations of PCR products, such as restriction digests, ligation, sequencing or hybridization, often require purification of the amplified DNA. Choose our PCR purification tools and gel extraction kits to obtain DNA fragments with the right purity and concentration for the next step. Merck Millipore offers a comprehensive array of centrifugal devices and MultiScreen® higher throughput filtration plates with which you can expect superior recovery, accuracy, and efficiency for your downstream molecular biology workflows.

SpinPrep™ PCR Clean-Up Kit

DNA binding to silica membrane and elution in an easy, spin-column format

Efficient primer removal with the SpinPrep™ PCR Clean-Up Kit



Lane	Sample
1	PCR Markers
2	Crude PCR product
3	Purified PCR product
4	PCR Markers

Features

- 10 min procedure
- Efficient removal of DNA polymerases, dNTPs, salts and primers
- Suitable for PCR purification of products for cloning, sequencing or labeling
- Up to 6 µg column binding capacity
- 100 bp-12 kb fragment clean up with up to 60-90% standard recovery

Description	Size	Catalogue No.
SpinPrep™ PCR Clean Up Kit	100 rxn	70976-3
Components:		
82 mL SpinPrep™ Bind Buffer 27 mL SpinPrep™ Wash Buffer 10 mL SpinPrep™ Elute Buffer 100 SpinPrep™ Filters 100 Receiver Tubes 100 SpinPrep™ Eluate Receiver Tubes		

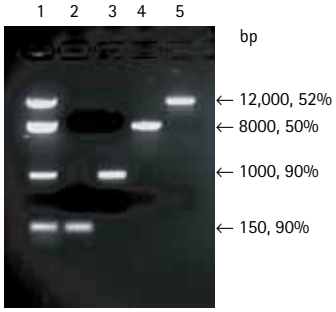
SpinPrep™ Gel DNA Kit

Rapid, efficient extraction of DNA from agarose gels

Features

- GelMelt™ Solution to dissolve gel slice followed by adsorption to silica membrane in spin column format
- No organic extraction or alcohol precipitation
- <30 min preparation
- No low melting point agarose requirement (150 mg gel slice/rxn)
- 150 bp to 12 kb fragment purification with 50-90% recovery
- 20 µg column binding capacity

Pure DNA fragments isolated with the SpinPrep™ Gel DNA Kit



2 mg of each DNA fragment were run in separate lanes on a 1% agarose gel. Each band was excised and the DNA extracted from the gel using the SpinPrep™ Gel DNA Kit. Recoveries shown as percentages were determined by absorbance at 260 nm. 250 ng of each recovered band were analyzed by agarose gel electrophoresis. Lane 1 contains a mixture of the starting (unpurified) DNAs.

Description	Size	Catalogue No.
SpinPrep™ Gel DNA Kit	100 rxn	70852-3
Components:		
5x24 mL SpinPrep™ GelMelt™ Solution 27 mL SpinPrep™ Wash Buffer 10 mL SpinPrep™ Elute Buffer 100 SpinPrep™ Filters 100 Receiver Tubes 100 SpinPrep™ Eluate Receiver Tubes		

Montage® Gel Extraction Kit

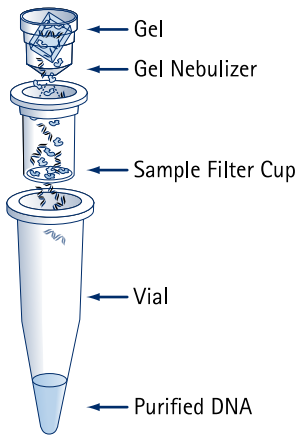
Centrifugal device for quickly purifying DNA from agarose gels

Features

- Minimal hands-on time
- 10 min spin
- Fully-functional DNA
- Suitable for cloning, radioisotopic or fluorescent labeled sequencing
- Validated for 100 bp-10 kb DNA size with 70% maximum recovery

Recover DNA from agarose gel slices in a single spin using this pre-assembled filter device. The unit is composed of a gel nebulizer, microcentrifuge vial, and modified TAE gel extraction buffer. Centrifugal force collapses the gel structure, driving the agarose through a small orifice in the nebulizer. The resultant gel slurry is collected into the sample filter cup and, for most applications, the DNA is ready for use.

Single-spin Operation



Exploded view of the Montage® gel extraction kit centrifugal device.

Description	Qty/Pk	Catalogue No.
Montage® Gel Extraction Kit	50	LSKGEL050*
Components: includes 50 Ultrafree®-DA devices and 500 mL of 50x concentrated modified TAE Buffer.		
Ultrafree®-DA Centrifugal Filter Unit	50	42600*
Modified TAE Buffer (50 x concentrated, 500 mL)	1	LSKMTAE50*

*Available from www.millipore.com

02

Meet Technical Demands in PCR Clean-Up and Nucleic Acid Preparation

Multiwell Nucleic Acid Sample Preparation

MultiScreen® PCR Filter Plates

Fast, automatable solutions for high throughput PCR purification

Features

- Automation compatible, fast, high-throughput solution
- No centrifugation or precipitation
- Excellent capacity, purity and recovery
- 99.5% primer removal

Based on size exclusion membrane technology and vacuum filtration, the MultiScreen® plates provide a one-step protocol with excellent results. No centrifugation or precipitaion steps are required. Sample recovery from the top of the plate makes it ideal for use with liquid handling systems. The plates are available in 96- and 384-well formats including a micro 96-well format for small volume PCR product purification (< 150 µL).

1. 2. 3.

Fast, high-throughput PCR clean-up in three easy steps:

1. Load PCR reactions.
2. Filter with Merck Millipore vacuum manifold for 5–10 minutes or until wells are dry.
3. Add water or buffer to each well. Agitate by shaking or pipetting. Retrieve purified samples by aspiration.

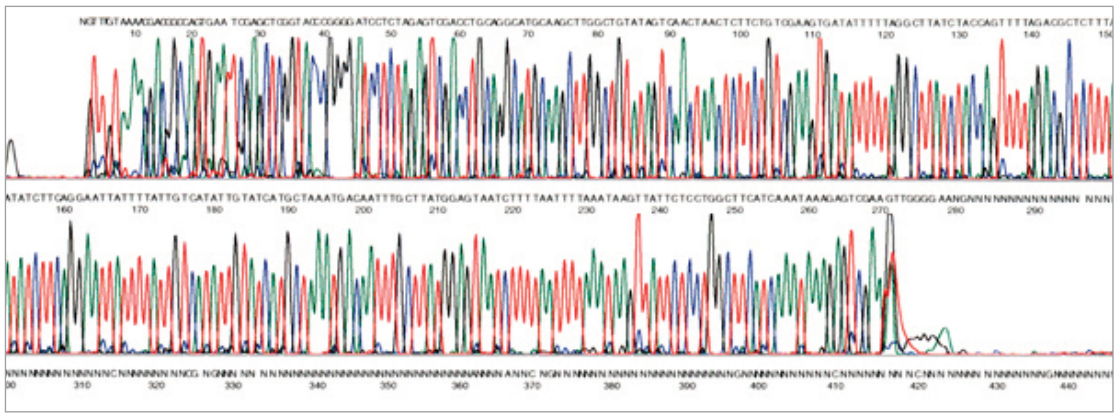
Description	Recommended Applications	Size	Catalogue No.
MultiScreen® $PCR_{\mu 96}$ filterplates (for 1-150 µL sample volumes)	PCR purification for: Cloning Sequencing Genotyping Microarray	10 50	LSKMPCR10* LSKMPCR50*
MultiScreen® $PCR_{\mu 96}$ filter Plates (for 150 µL-300 µL sample volumes)		10 50	MSNU03010* MSNU03050*
MultiScreen® PCR_{384} Filter Plates (1-100 µL sample volumes)		10 50	S384PCR10* S384PCR50*

*Available from www.millipore.com

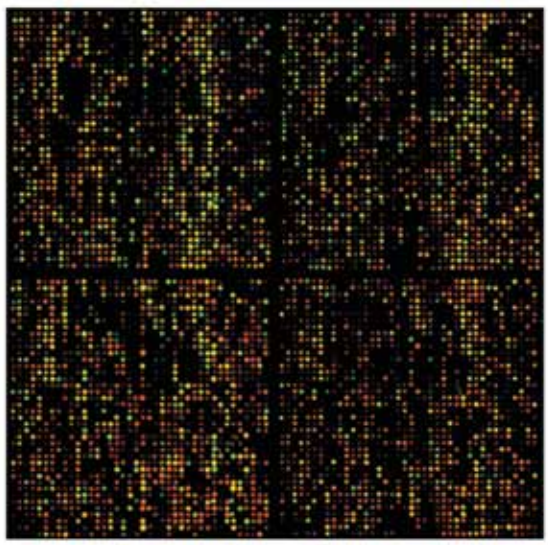
Meet Technical Demands in PCR Clean-Up and Nucleic Acid Preparation

Multiwell Nucleic Acid Sample Preparation

MultiScreen® purification enables long sequencing reads (A) and informative microarray hybridization (B).



A. Representative electropherogram from a 301 bp PCR product purified using MultiScreen® PCR_{96} filter plate (Thermo Sequenase® II dye terminator).



B. Enlarged section of a 19,299 element microarray (image reproduced with permission from Dr. John Quackenbush, The Institute for Genomic Research). High quality microarrays can be manufactured using PCR products purified with MultiScreen® PCR products. Reference: Hegde, P., R. Qi, K. Abernathy, C. Gay, S. Dhaarp, R. Gaspard, J.E. Hughes, E. Snesrud, N. Lee and J. Quackenbush. 2000. A concise guide to cDNA microarray analysis. BioTechniques 29: 548–562.

Accessories

Description	Qty/Pk	Catalogue No.
MultiScreen® Vacuum Manifold (96-well)	1	MAVM0960R*
MultiScreen®$_{384}$ Vacuum Manifold (µ96- and 384-well)	1	SAVM38401*

*Available from www.millipore.com

Montage® Miniprep 96-well Kit

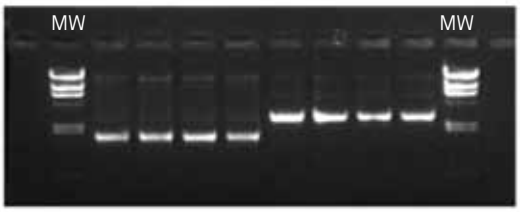
Fast, easy purification of plasmids and BACs (bacterial artificial chromosomes)

Features

- No centrifugation or precipitation steps
- Minimum processing times
- Excellent purity, yields and reproducibility
- Automation compatible

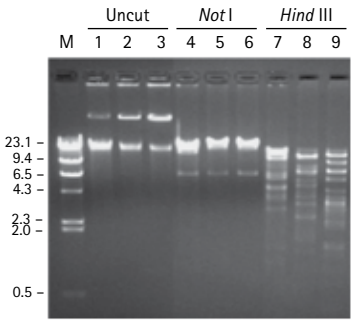
A patented separation technology offers a simple protocol eliminating lengthy bind/elute and centrifugation methods. Clean and reproducible DNA is purified in 50% less time than traditional methods. Following bacterial lysis, 3 short filtration steps allow preparation of DNA in a 96-well format. The collection of samples from the top of the plates enhances the kit's suitablility with a wide range of automated liquid handling systems.

Superior quality plasmid purification



Agarose gel showing quality and consistency of plasmid DNA purified usir the Montage® Plasmid Miniprep$_{HTS}$ 96 Kit (MW-lambda Hind III digest).

Excellence in BAC purification



Three different BAC clones from a human chromosome 22 human BAC DNA library (Research Genetics, Inc.) were purified with the Montage® Plasmid Miniprep$_{HTS}$ 96 Kit and electrophoresed on a 0.8% agarose gel for 120 min at 100 V either uncut (lanes 1 – 3) or after digestion with Not I (lanes 4 – 6) or Hind III (lanes 7 – 9). Lane M is a Hind III digest of Lambda phage DNA. The BAC DNA purified with the Montage® Plasmid Miniprep$_{HTS}$ 96 Kit is suitable for fingerprinting.

Description	Qty/Pk	Catalogue No.
Montage® Miniprep96 kit	4	LSKP09604*
	24	LSKP09624*
MultiScreen®$_{HTS}$-NA for lysate clearing	10	MSNANLY10*
	50	MSNANLY50*
The kit contains all the reagents and disposable materials you need to purify plasmid DNA in a 96-well format.		

*Available from www.millipore.com

Montage® SEQ$_{96}$ Sequencing Reaction Clean-Up Kit

High-throughput filter microplates with patented size-exclusion technology to yield highly purified sequencing reaction products

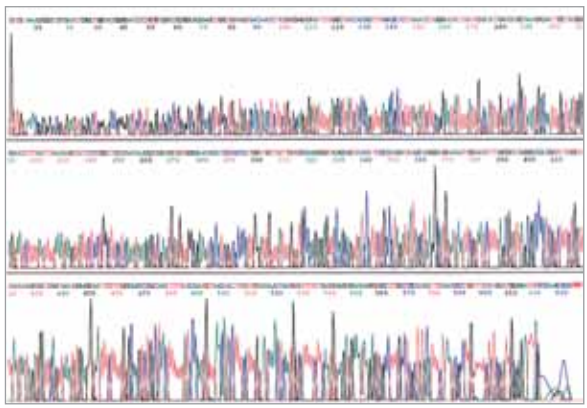
Features

- Highly efficient 10 min vacuum-based protocol
- Compatible with a variety of templates
- Optimized for BigDye® Chemistries
- Size exclusion purification eliminates variability in sequence reaction clean-up
- 96- & 384-well format
- Automation-compatible

To generate high quality DNA sequence data, unincorporated dye terminators and salts must be removed from sequencing products prior to capillary and slab gel electrophoresis. The uniform desalting and recovery capabilities of the Montage® SEQ$_{96}$ filter plate eliminate capillary-to-capillary variability that is observed with ethanol precipitation.

In addition to eliminating centrifugation, the plates do not require filtrate collection or column packing. The vacuum-driven protocol is designed for use with automated DNA sequencers, including ABI PRISM® and MegaBACE® systems. Since the membrane-based protocol does not require alcohol precipitation, there is no risk of salts or ethanol affecting final sequencing results. In addition to high pass rates, highly reproducible results are obtained with PCR and plasmid templates prepared by a variety of methods.

Purify sequencing reactions from PCR templates with the Montage® SEQ$_{96}$ Sequencing Reaction Clean-Up Kit.



A 657 bp PCR product (10 fmole) was purified using the Montage® PCR$_{\mu 96}$ filter plate and sequenced in a 1/8 X BigDye® Terminator v1.1 reaction. After purification using the Montage® SEQ$_{96}$ Sequencing Reaction Cleanup Kit, the samples were analyzed on an ABI PRISM® 3700 DNA sequencer. Using this approach, the first base after the primer is readily detectable.

Description	Qty/Pk	Catalogue No.
Montage® SEQ$_{96}$ Sequencing Reaction Clean-up Kit	1	LSKS09601
Montage® SEQ$_{96}$ Sequencing Reaction Clean-up Kit	4	LSKS09604
Montage® SEQ$_{96}$ Sequencing Reaction Clean-up Kit	24	LSKS09624
Components: all kits include 96-well filter plates and injection solution.		
MultiScreen® SEQ$_{384}$ filter Plates (reagents not included)	10 50	S384SEQ10* S384SEQ50*
Accessories:		
Montage® Wash Solution Montage® Injection Solution	1x 500 mL 1x 500 mL	LSKSBW500* LSKSIS500*

*Available from www.millipore.com

Pellet Paint® Co-Precipitant

A visible dye-labeled carrier of nucleic acids for rapid, quantitative nucleic acid precipitation

Features:

- Direct visualization and tracking of alcohol precipitated nucleic acids
- Efficient precipitation of DNA or RNA from dilute solutions (2 ng/mL)
- Prevents loss of DNA or RNA pellets
- Compatible with most procedures including Cy5® sequencers. Recommended for Applied Biosystems automated sequencers.

Pellet Paint® co-precipitant is compatible with more applications compared to other carriers for nucleic acid precipitation

Compatible with	Pellet Paint	glycogen	tRNA
gel electrophoresis	✔	✔	–
PCR amplification	✔	?	–
DNA sequencing	✔	✔	–
restriction digestion	✔	✔	✔
ligation	✔	✔	?
transformation	✔	✔	?
cDNA synthesis	✔	✔	?
kinase reactions	✔	✔	–
random priming	✔	?	–
in vitro transcription	✔	✔	?
in vitro translation	✔	✔	✔
RNase protection assay	✔	?	✔
phenol extraction	✔	✔	✔
LiCl precipitation	✔	✔	–
bacterial electroporation	✔	?	?
PEG precipitation	✔	?	?

Pellet Paint® Co-precipitant enhances recovery of RNA and DNA preparations

Sample	% recovery (% cpm incorporated)
RNA (100 nt, 0.2 ng/mL)	90%
RNA (1000 nt, 0.2 ng/mL)	92%
RNA (10,000 nt, 0.2 ng/mL)	89%
DNA (100-2000 bp, 4 pg/mL)	86%

The samples of 32P-labeled RNA and DNA were prepared using standard protocols for transcription and random priming, respectively. Following the labeling reactions, incorporation was determined by DE81 filtration. Known amounts of incorporated material (300,000 cpm) were precipitated in the presence of Pellet Paint®. Samples without Pellet Paint® Co-Precipitant resulted in a 5- to 50-fold reduction in recovery.

Pellet Paint® Co-Precipitant

Pellet Paint® pellet (2 mL) under UV and visible illumination

Description	Size	Catalogue No.
Pellet Paint® Co-Precipitant	125 rxn 1,000 rxn	69049-3 69409-4
Components:		
250 µL or 2 mL Pellet Paint® Co-Precipitant 1 mL or 8 mL of 3M Sodium Acetate pH 5.2		

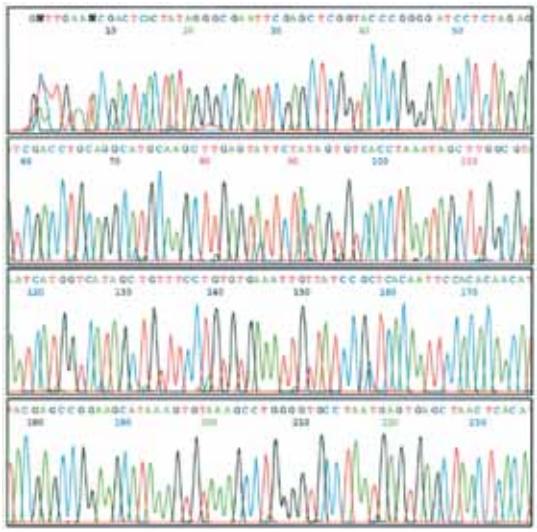
Pellet Paint® NF Co-Precipitant

Non-fluorescent, visible nucleic acid co-precipitant for automated sequencing applications

Features

- Non-fluorescent dye-labeled carrier
- Efficient and rapid precipitation of BigDye® cycle sequencing products
- Efficient removal of dye terminators
- Direct visualization and tracking of precipitated material
- No effect on sequencing reaction
- Compatible with fluorescent detection systems

Cycle sequencing with Pellet Paint® NF Co-Precipitant



Pellet Paint® NF co-precipitant (2 µL)* was combined with 100 ng plasmid and 1.6 pmol primer in 10 mL BigDye® Terminator Cycle Sequencing Reaction performed in 8-well Micro- Amp® reaction tubes. Reaction products mixed with isopropanol and centrifuged for 10 min at 3000 × g. Samples were drained by inversion and the inverted plate was spun at low force. The samples were resuspended in 20 µL Template Suppression Reagent and run on an ABI PRISM® 310 sequencer. Sequence data were identical to those obtained with a control reaction without Pellet Paint® NF (not shown).
* Note that the standard precipitation reaction uses 1 µL.

Description	Size	Catalogue No.
Pellet Paint® NF Co-Precipitant	125 rxn 1000 rxn	70748-3 70748-4
Components:		
125 µL or 1 mL Pellet Paint® NF Co-precipitant 1 mL or 8 mL 3M Sodium Acetate pH 5.2		

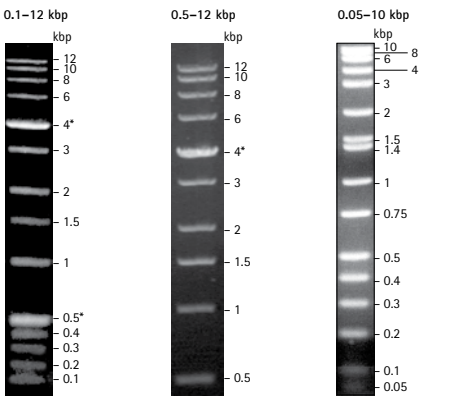
Molecular Size Markers

Perfect DNA™ Markers

Accurate sizing for DNA gel electrophoresis, in easy formats

Description	Size Range	Catalogue No.
Perfect DNA™ Markers (100 lanes)	0.05-10 kb 0.1-12 kb 0.5-12 kb	69002-3 70087-3 70540-3
Components:		
500 µL DNA markers (1x DNA gel loading buffer) 1 mL 6x DNA Gel Loading Buffer		
Features:		
• Uniform band intensities except for the easily identifiable reference bands • Easy-to-remember sizes		
Perfect DNA™ Ladders (100 lanes)	50-300 bp 100-1000 bp 500-10,000 bp	70538-3 70539-3 70537-3
Components:		
1 mL of DNA ladder in 1x loading buffer 1 mL of 6x loading buffer (or 6x DNA gel loading buffer-70537)		
Features:		
• Evenly spaced, uniform band intensities • Wide range of DNA sizes • Ready-to-load format		
PCR Markers (50 lanes)	50-2000 bp	69278-3
Components:		
250 µL PCR Markers in 1x loading buffer 1 mL 6x Loading Buffer		
Features:		
Features: • Brightly spaced bands with convenient size ranges for small DNA products • Ready to use in gel loading buffer with tracking dyes for monitoring electrophoresis		

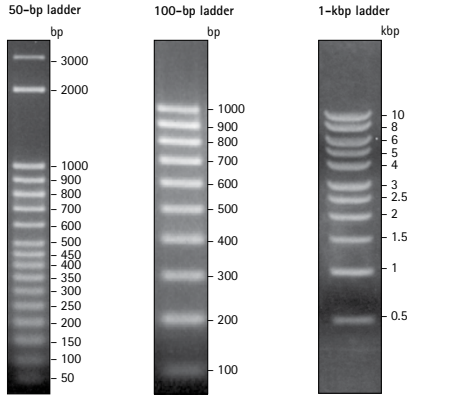
Perfect DNA™ Markers



1.5% TAE agarose gel* | 0.8% TAE agarose gel* | 2.0% TAE agarose gel

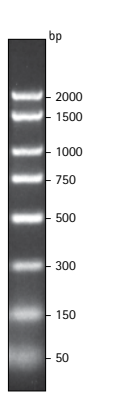
*higher-intensity reference band

Perfect DNA™ Ladders



2.0% TAE agarose gel | 2.0% TAE agarose gel | 0.8% TAE agarose gel

PCR Markers



1.5% TAE agarose gel

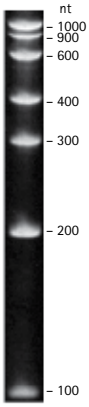
Molecular Size Markers

Perfect RNA™ Markers

Wide range of exact size RNA markers qualified for native or denaturing gels

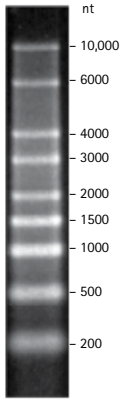
Description	Size Range	Catalogue No.
Perfect RNA™ Markers	0.1-1 kb	69924-3
Features:		
• Mixture of 7 defined RNA transcripts • Uniform band intensities and easy-to-remember size patterns • For native or denaturing agarose or polyacrylamide gel analysis of small RNA, especially RNase protection assays		
Perfect RNA™ Markers	0.2-10 kb	69946-3
Features:		
• Mixture of 9 defined RNA standards under native or denutauration conditions • Convenient interval marking and easy-to-remember sizes • Accurate size determination and quality analysis of RNA preparations and *in vitro* transcription products		

Perfect RNA™ Markers,
0.1-1 kb,



5% polyacrylamide-urea gel

Perfect RNA™ Markers,
0.2-10 kb



1% TBE agrose gel

03 Preserve, Propagate and Express

Fast Protocol PCR Cloning Kits

Merck Millipore cloning tools are designed with simplicity, efficiency and fast workflows in mind. Our solutions include kits for subcloning PCR fragments for easy preservation and propagation of clones, ensuring a sufficient supply of target for downstream applications. In addition, we offer streamlined solutions combining simplified cloning tools with direct protein expression platforms, based on the globally renowned vector expertise of Novagen®.

Guide to Cloning Kits: Find the Best Fit Solution for your Workflow

Product	Vector	Application	Vector features
AccepTor™ Vector Kit (for PCR products from non-proofreading DNA polymerase	pSTBlue-1	Archiving Subcloning Sequencing *In vitro*-transcription	SP6/T7 promoters Blue/White screening Amp & Kan selection Dual EcoR1 sites
	pETBlue™-1	Protein Expression	No fusion tags Blue/White screening IT7/lac-driven, tightly controlled, high-level expression in *E.coli*
Perfectly Blunt® Cloning Kits (any DNA polymerase product since kit includes end conversion mix for blunt DNA)	pSTBlue-1	Archiving Subcloning Sequencing *In vitro*-transcription	SP6/T7 promoters Amp & Kan selection Dual EcoR1 sites
	pT7Blue-3		T7 promoter Amp & Kan selection Dual EcoR1 sites
	pT7Blue		T7 promoter NdeI/BamHI sites flank insert T7 driven *In vitro* protein synthesis
	pT7Blue-2	Protein Expression *In vitro* transcription/translation Sequencing	N-terminal S•tag sequence Optimal Kozak translation initiation *Xenopus* globin 5'UTR
	pETBlue-1	Protein Expression	T7 promoter Optional C-terminal HSV•Tag® sequences Vector has ATG start codon
	pETBlue-2		

AccepTor™ Vector Kits

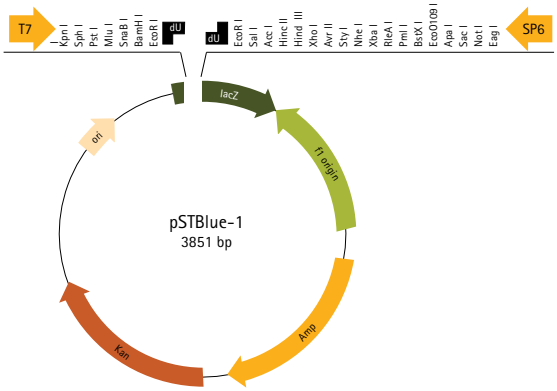
Rapid, direct cloning with TA cloning technology

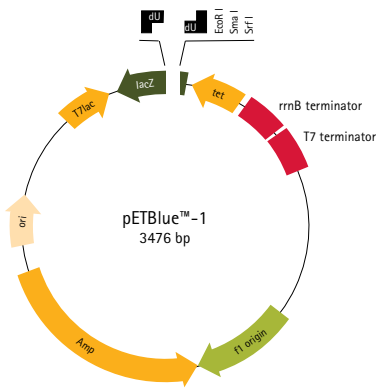
Features:

- Fast and simple protocol; 40 min from PCR to plating transformants
- No need for restriction digestion or special primers
- Direct ligation of PCR product with vector
- Blue/White Screening with pSTBlue-1 or pETBlue™-1 vectors- up to 80% recombinant
- Compatible with products from polymerases that leave single 3'-dA overhang

AccepTor™ Kit Vectors contain cloning sites with single 3'-dU ends for direct ligation of PCR products with single 3'dA ends without the need for intermediate reactions. Following transformation the dU residues are converted to dT residues *in-vivo*.

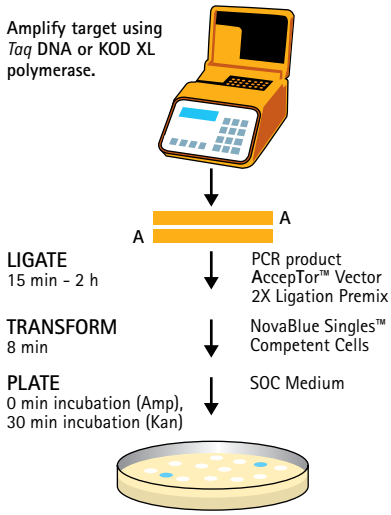
The AccepTor™ Vector kits contain linearized vector ready to mix with PCR product and Clonables™ 2x Ligation Premix. The ligation reaction is subsequently transformed into pre-aliquoted NovaBlue Singles™ competent cells. AccepTor™ GigaSingles™ Kits contain extremely high efficiency NovaBlue competent cells for demanding procedures. For pETBlue™-1 kits, the initial cloning is performed in the non-expression host NovaBlue, and the recombinant plasmid is transformed into Tuner™(DE3)pLacI Competent Cells (included in kit) for efficient protein expression.





AccepTor™ Vectors: pSTBlue-1 (left) is a general purpose vector. pETBlue™-1 (right) is a high copy number expression plasmid.

AccepTor™ Vector Kit protocol and primer design



Primer design for pETBlue-1 AccepTor™ Vector:
Use pETBlue-1 vector to express unfused proteins from inserts that have an ATG start codon. The AccepTor™ cloning site is located just downstream of the T7 gene 10 RBS (ribosome binding site). Amplify the insert using primers as shown:

Sense Primer:	**Met... 5'-ATGXXX...**
Antisense primer:	**No restrictions**

AccepTor™ Vector Cloning and Giga Cloning Kit Configurations

	Introductory Kits	AccepTor™ Vector Kits		AccepTor™ Vector		pSTBlue-1 AccepTor™ Vector Giga Cloning Kits	
Kit Component	10 rxn	20	40 rxn	20 rxn	40 rxn	20 rxn	40 rxn
AccepTor™ Vector	0.5 mg	2 × 0.5 mg	4 × 0.5 mg	2 × 0.5 mg	4 × 0.5 mg	2 × 0.5 mg	4 × 0.5 mg
Positive Control Insert	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
Clonables™ 2X Ligation Premix	55 mL	2 × 55 mL	4 × 55 mL			2 × 55 mL	4 × 55 mL
Nuclease-free Water	1.5 mL	1.5 mL	1.5 mL			1.5 mL	1.5 mL
NovaBlue Singles™ Competent Cells*	11 × 50 mL	22 × 50 mL	44 × 50 mL			22 × 50 mL	44 × 50 mL
SOC Medium†	2 (or 3) × 2 mL	4 (or 5) × 2 mL	7 (or 9) × 2 mL			4 × 2 mL	7 × 2 mL
Test Plasmid	10 mL	10 mL	10 mL			10 mL	10 mL

* The pETBlue AccepTor™ Vector Kits also contain Tuner™(DE3)pLacI Competent Cells (0.2 mL for 10 rxn, 2 × 0.2 mL for 20 rxn, and 4 × 0.2 mL for 40 rxn). The AccepTor™ Vector Giga Cloning Kits subsitute NovaBlue GigaSingles™ for NovaBlue Singles™ Competent Cells.

† The pETBlue AccepTor™ Vector Kits contain extra SOC Medium, as indicated in parentheses.

Description	Size	Catalogue No.
pSTBlue-1 Kits & DNA		
Introductory pSTBlue-1 AccepTor™ Vector Kit	10 rxn	70594-3
pSTBlue-1 AccepTor™ Vector Kit	20 rxn	70595-3
pSTBlue-1 AccepTor™ Vector Kit	40 rxn	70595-4
pSTBlue-1 AccepTor™ Vector Giga Kit	20 rxn	71228-3
pSTBlue-1 AccepTor™ Vector Giga Kit	40 rxn	71228-4
pSTBlue-1 AccepTor™ Vector Giga Kit	40 rxn	70596-4
pSTBlue-1 AccepTor™ Vector (linearized vector)	20 rxn	70596-3
pSTBlue-1 AccepTor™ Vector (linearized vector)	40 rxn	70596-4
pSTBlue-1 DNA	20 µg	70199-3
pETBlue-1 Kits & DNA		
Introductory pETBlue™-1 AccepTor™ Vector Kit	10 rxn	70597-3
pETBlue-1 AccepTor™ Vector Kit	20 rxn	70598-3
pETBlue-1 AccepTor™ Vector Kit	40 rxn	70598-4
pETBlue-1 AccepTor™ Vector (linearized vector)	20 rxn	70599-3
pETBlue-1 AccepTor™ Vector (linearized vector)	40 rxn	70599-4
pETBlue™-1 DNA	20 µg	70608-3

Perfectly Blunt® Cloning Kits

Efficient cloning of DNA amplified by any polymerase

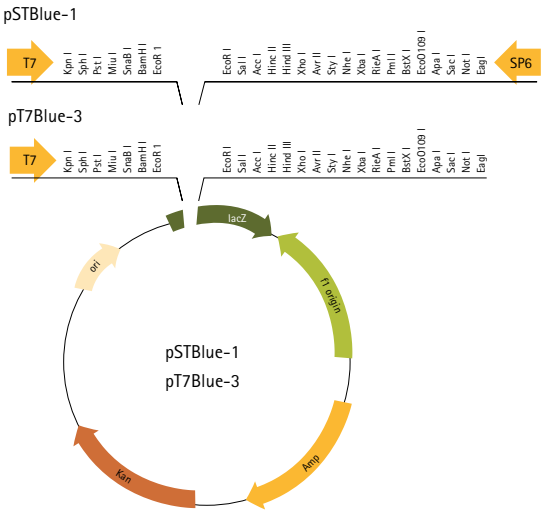
Features

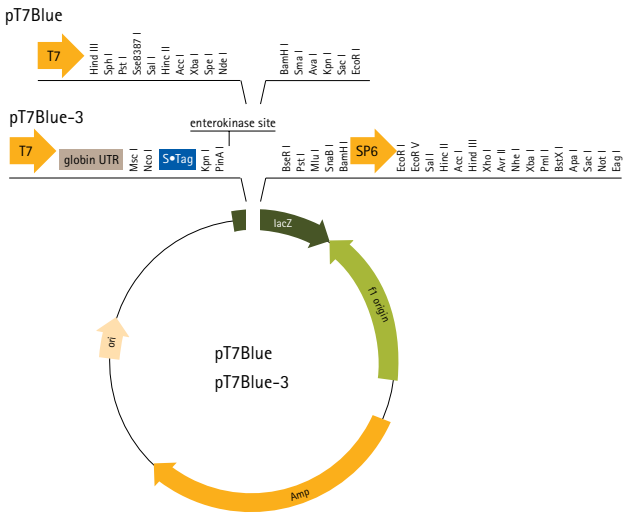
- Multiple vector (including pETBlue™ vector) options to suit application needs
- No restriction enzymes or special primers required
- Compatible with inserts generated by any DNA Polymerase (end conversion mix included in kit)
- Blue/White screening
- Fast protocol: <1 hour from PCR product to plating transformants
- Up to 95% recombinants
- Suitable for cloning restriction fragments, cDNA or sheared DNA

Perfectly Blunt® Cloning Kits simplify highly efficient cloning of PCR products generated with any thermostable DNA Polymerase, enabling you to use high fidelity, proofreading DNA polymerases for amplification to decrease the probability of generating mutations in the target sequence.

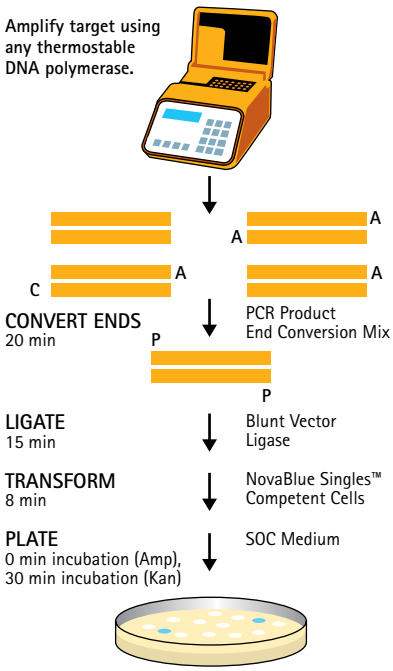
In the Perfectly Blunt® method, PCR products are converted to blunt, phosphorylated DNA in a 15 minute reaction using premixed reagents. After a 5-minute heat inactivation step, the treated insert is combined with ready-to-use vectors (pT7, pST and pET options, as shown). Following an optimized 15 minute ligation, an exclusive 8-minute transformation procedure using NovaBlue Singles™ Competent Cells generates recombinant colonies with high efficiency.

Perfectly Blunt® vector maps with indicated multiple cloning sites





Perfectly Blunt® cloning protocol and primer design

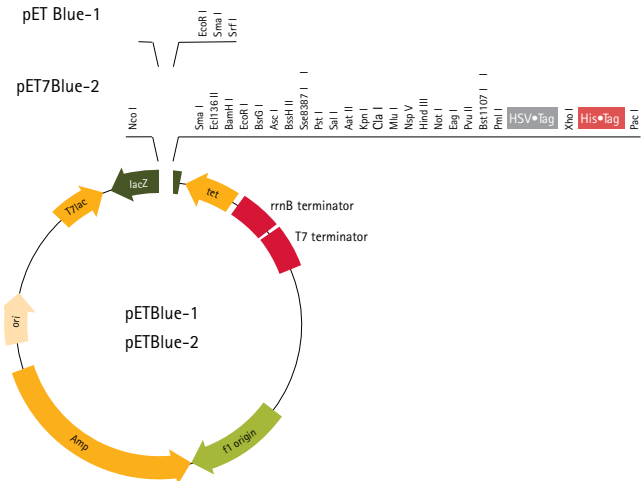


Primer design for pT7Blue-2 Perfectly Blunt® Vector

Use pT7Blue-2 for T7 promoter-driven expression in reticulocyte lysate or lac promoter-driven expression in E. coli of proteins fused with an upstream, cleavable S•Tag™ sequence. Amplify your insert with primers as shown:

Sense Primer:	**5'-NXXX...***
Antisense primer:	**No restrictions**

*where N = any base (completes Pro codon; G or A is recommended) and XXX = the initial codon of the insert.
Note: For additional information and to download a copy of User Protocol TB183, visit www.merckbiosciences.com



Primer design for expression of inserts in pETBlue™-1 and pETBlue™-2 Blunt Vectors

Use pETBlue™-1 vector to express unfused proteins from inserts that have an ATG start codon. Amplify your insert using primers as shown:	Use pETBlue-2 to express proteins fused with C-terminal HSV•Tag® and His•Tag® sequences to facilitate detection and purification. Amplify your insert using primers as shown:
Met... Sense Primer: **5'-ATGXXX...** Antisense primer: **No restrictions**	Vector: **MetAlaIle[insert]Ser** **ATGGCGATNXXX.....ATCC** **TACCGCTA.....YYYNNTAGG** Sense Primer: **5'-NXXX.....** If N = G, Met codon is generated instead of Ile. Antisense primer*: **5'-NNYYY.....** *If NN = CA or TA, stop codon is generated in sense strand.

Additional Information: Perfectly Blunt® Cloning Kit User Protocol TB183 pETBlue™ System Manual TB249. To download copies of these protocols, visit www.merckbiosciences.com

Fast Protocol PCR Cloning Kits

Perfectly Blunt® Cloning and Giga Cloning Kit Configurations

	Introductory Kits	Perfectly Blunt® Cloning Kits		Blunt Vector Kits		pSTBlue-1 Perfectly Blunt® Giga Cloning Kits	
Kit Component	10 rxn	20 rxn	40 rxn	20 rxn	40 rxn	20 rxn	40 rxn
Blunt Vector	0.5 mg	2 × 0.5 mg	4 × 0.5 mg	2 × 0.5 mg	4 × 0.5 mg	2 × 0.5 mg	4 × 0.5 mg
Positive Control Insert	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
End Conversion Mix	100 mL	100 mL	2 × 100 mL	100 mL	2 × 100 mL	100 mL	2 × 100 mL
T4 DNA Ligase	100 U	100 U	2 × 100 U			100 U	2 × 100 U
Nuclease-free Water	1.5 mL	1.5 mL	1.5 mL			1.5 mL	1.5 mL
NovaBlue Singles™ Competent Cells*	11 × 50 mL	22 × 50 mL	44 × 50 mL			22 × 50 mL	44 × 50 mL
SOC Medium†	2 (or 3) × 2 mL	5 (or 6) × 2 mL	7 (or 9) × 2 mL			4 × 2 mL	7 × 2 mL
Test Plasmid	10 mL	10 mL	10 mL			10 mL	10 mL

* The pETBlue Perfectly Blunt® Cloning Kits also contain Tuner(DE3)pLacI Competent Cells. The Perfectly Blunt® Giga Cloning Kits subsitute NovaBlue GigaSingles™ for NovaBlue Singles™ Competent Cells.

† The pETBlue Perfectly Blunt® Vector Kits contain extra SOC Medium, as indicated in parentheses.

Description	Size	Catalogue No.
pSTBlue-1 Kits & DNA		
Introductory pSTBlue-1 Perfectly Blunt® Cloning Kit	10 rxn	70184-3
pSTBlue-1 Perfectly Blunt® Cloning Kit	20 rxn	70191-3
pSTBlue-1 Perfectly Blunt® Cloning Kit	40 rxn	70191-4
pSTBlue-1 Blunt Vector (linearized)	20 rxn	70188-3
pSTBlue-1 Blunt Vector (linearized)	40 rxn	70188-4
pSTBlue-1 Perfectly Blunt® Giga Cloning Kit	20 rxn	71229-3
pSTBlue-1 DNA	20 µg	70199-3
pT7Blue-3 Kits & DNA		
Introductory pT7Blue-3 Perfectly Blunt® Cloning Kit	10 rxn	70075-3
pT7Blue-3 Perfectly Blunt® Cloning Kit	20 rxn	70182-3
pT7Blue-3 Perfectly Blunt® Cloning Kit	40 rxn	70182-4
pT7Blue-3 Blunt Vector (linearized)	20 rxn	70187-3
pT7Blue-3 DNA (uncut)	20 µg	70025-3
pT7Blue-2 Kits & DNA		
pT7Blue-2 Blunt Vector (linearized)	20 rxn	70186-3

Clonables™ Ligation/Transformation Kit

Simple, reproducible ligation and transformation

Features

- Rapid 15 min ligation and 8 minute transformation with ampicillin selection
- Convenient, premixed ligation reagents for reproducibility with minimal pipetting
- One reaction condition, optimized for sticky ends, single-base overhangs and blunt ends
- Single-use competent cells eliminate aliquoting, freeze/thaw and waste of partially-used vials
- Compatible with PCR buffer, TE, restriction enzyme buffer and End Conversion Mix

The Clonables™ Kit enables convenient, dependable, high-efficiency ligation compatible ends and efficient transformation into competent cells. The unique, universal ligation mix contains ligase, buffer and cofactors to support ligation of any type of DNA sticky or blunt ends in a 15 minute reaction. The streamlined transformation protocol takes less than 8 minutes for ampicillin resistant plasmids or <40 minutes for other antibiotic-resistant plasmids. The flexible kit works with a variety of cloning vectors without requiring additional modification of cloning junctions or DNA ends.

Description	Size	Catalogue No.
Clonables™ Ligation/Transfromation Kit	11 rxn	70526-3
Components:		
Clonables™ 2X Ligation Premix	55 µL	
Clonables™ Positive Control	10 µL	
Nuclease-free Water	1.5 mL	
NovaBlue Singles™ Competent Cells	11 × 50 µL	
SOC Medium	2 × 2 mL	
Test Plasmid	10 µL	
Premix available separately:		
Clonables™ 2X Ligation Premix	55 µL	70573-3
Clonables™ 2X Ligation Premix	2.5 mL	70573-4

03

Preserve, Propagate and Express

General Competent Cells for Cloning

For successful isolation and propagation of engineered DNA vectors, transformation into competent bacterial cells is a key step. The right strain of competent cells can make the difference between cloning frustration and progressing to the next step. Merck Millipore offers a range of strains and formats to suit most cloning projects. Since reliability is critical, we verify the phenotype and purity of each strain and guarantee its transformation efficiency. With more than two decades of experience producing competent cells for routine cloning and protein expression we offer robust performance you can count on.

NovaBlue™ Competent Cells

This K-12 strain is ideally suited as an initial cloning host attributed to its high transformation efficiency, blue/white screening capability (with appropriate plasmids), recA and endA resulting in excellent yields of high quality plasmid DNA.

Formats include:

- Singles™ format (50 µL single use tubes)
- High-throughput HT96™ format (20 µL volume in 96-well plate)
- Standard format (0.2 mL volume sufficient for ten transformations)
- Veggie Singles™, prepared with animal-free reagents for applications in which animal-derived products are prohibited

NovaBlue™ Competent Cell Formats

	Transformation Efficiency	Reaction Size	Application
GigaSingles™	> 1.0 × 10^9 cfu/µg	50 µL	High-efficiency cloning
Singles™	> 1.5 × 10^8 cfu/µg	50 µL	Routine cloning
Veggie Singles™	> 1.5 × 10^8 cfu/µg	50 µL	Applications requiring non-animal-derived materials Routine cloning
HT96™	> 1.0 × 10^8 cfu/µg	96 x 20 µL	High-throughput cloning
Standard	> 1.5 × 10^8 cfu/µg	20 µL	Routine cloning

NovaBlue Genotype:
endA1 hsdR17(r_{K12}^- m_{K12}^+) supE44 thi-1 recA1 gyrA96
relA1 lac F'[$proA^+B^+$ $lacI^qZ\Delta M15$::Tn10] (Tet^R)

NovaBlue GigaSingles™ Competent Cells

10^9 efficiency in chemically competent cells

Features

- Guaranteed efficiency > 1x 10^9 cfu/µg
- Ideal for high efficiency cloning
- Enables production of high-quality plasmid DNA
- Single-use formats
- Prepared using an optimized chemical method

Description	Size	Catalogue No.
NovaBlue GigaSingles™ Competent Cells	11 rxn	71227-3
NovaBlue GigaSingles™ Competent Cells	22 rxn	71227-4
Components:		
Competent Cells	11 x 50 µL or 22 x 50 µL	
SOC Medium	2 x 2 mL or 4 x 2 mL	
Test Plasmid	10 µL	

NovaBlue Singles™ Competent Cells

Features

- Guaranteed efficiency >1.5 x 10^8 cfu/µg
- Single-use formats
- Enables high-quality plasmid DNA preparation
- Rapid: Transformation directly into the tube

NovaBlue Singles™ Competent Cells, like all Novagen® Singles Competent Cells, are designed for ultimate convenience and reliability in plasmid transformation. The cells are grown and made chemically competent using an optimized procedure. Pre-aliquoted (50 µL) volumes minimize freeze/thaw or waste of partially used vials. The Singles™ format can save you money and ensures reliable cell performance.

Description	Size	Catalogue No.
NovaBlue Singles™ Competent Cells	11 rxn	70181-3
NovaBlue Singles™ Competent Cells	22 rxn	70181-4
Components:		
Singles™ Competent Cells	11 x 50 µL or 22 x 50 µL	
SOC Medium	2 x 2 mL or 4 x 2 mL	
Test Plasmid	10 µL	

NovaBlue T1^{R} Singles™ Competent Cells

All the qualities of NovaBlue cells with added benefit of resistance to T1 and T5 Phage

Features

- Guaranteed efficiency >1.5 x 10^{8} cfu/µg
- Resistant to T1 and T5 phage
- Enables production of high-quality plasmid DNA
- Easy-to-use Singles™ format
- Prepared using an optimized chemical method

The NovaBlue T1^{R} strain has the same features as NovaBlue, with the added benefit of being resistant to T1 and T5 phage.

Description	Size	Catalogue No.
NovaBlue T1^{R} Singles™ Competent Cells	22 rxn	71318-4
Components:		
NovaBlue T1^{R} Singles™ Competent Cells	22 x 50 µL	
Veggie™ SOC Medium	4 x 2 mL	
Test Plasmid	10 µL	

NovaBlue T1^{R} Genotype:
endA1 hsdR17(r_{K12}^{-} m_{K12}^{+}) supE44 thi-1 recA1 gyrA96 recA1 lac tonA F'[$proA^{+}B^{+}$ $lacI^{q}ZΔM15$::Tn10] (Tet^{R})

Veggie™ NovaBlue Singles™ Competent Cells

Certified animal-free NovaBlue competent cells

Features

- Guaranteed efficiency >1.5 x 10^{8} cfu/µg
- Manufactured free of animal-derived media and components
- Reproducible high-efficiency cloning
- Enables production of high-quality DNA
- Easy-to-use Singles™ format
- Prepared using an optimized chemical method

Take advantage of the features and benefits of NovaBlue Singles™ prepared with animal-free reagents for applications in which animal-derived products are prohibited.

Description	Size	Catalogue No.
Veggie™ NovaBlue Singles™ Competent Cells	11 rxn	71251-3
Veggie™ NovaBlue Singles™ Competent Cells	22 rxn	71251-4
Components:		
Veggie™ NovaBlue Singles™ Competent Cells	11 x 50 µL or 22 x 50 µL	
Veggie™ SOC Medium	2 x 2 mL or 4 x 2 mL	
Test Plasmid	10 µL	

HT96™ NovaBlue Competent Cells

Transformations for high-throughput applications

Features

- Guranteed efficiency > 1 x 10^8 cfu/µg
- Pre-aliquoted in specialized 96-well format

For your high-throughput needs, NovaBlue Competent cells are offered as pre-dispensed 20 µL volumes in a 96-well polypropylene plate compatible with a variety of thermal cyclers and water baths. Wells are individually sealed with raised rims to avoid cross-contamination. The cells can be accessed directly by piercing seals with standard pipet tip or easy seal removal. Strips of caps are also provided for reliable sealing during manipulation or storage. For lower throughput processing, groups of 24 wells can be easily split from the 96 well plate.

Description	Quantity	Catalogue No.
HT96™ NovaBlue Competent Cells	1 plate	71011-3
HT96™ NovaBlue Competent Cells	4 plates	71011-4
Components:		
HT96™ NovaBlue Competent Cells	1 or 4 plates	
SOC Medium	1 x 14 mL or 4 x 14 mL	
Test Plasmid	1 x 10 µL or 2 x 10 µL	
8-Cap Strips	1 or 4 pkg	
Reagent Reservoirs	1 or 4	

HT96™ Thermal Block

Efficient thermal transfer to samples in HT96™ plate

Features

- Anodized aluminium, solvent resistant block to hold one HT96™ plate
- Provides efficient thermal transfer to samples held within 96-well plate
- Allows rapid transfer of samples between low temperature and heat shock steps in transformation protocols.
- Simple pre-incubation of thermal block prior and then place HT96™ competent cell plate in block
- Compatible with most 96-well and robotic platforms

Description	Quantity	Catalogue No.
HT96™ Isothermal Block	1 ea	71195-3

04 Essentials for Your Molecular Biology Toolkit

Molecular Biology Grade Buffers and Enzymes

OmniPur® Agarose PCR Plus
(Catalogue No. 2010)

For superior resolution of DNA fragments, especially small fragments (<1000 bp and PCR products), use OmniPur® Agarose PCR Plus. OmniPur® Products represent a grade of molecular biology reagents that are of the highest quality and deliver consistent performance from lot to lot. Each lot of OmniPur® grade reagents is tested for the absence of DNase, RNase, and protease for safe use in tissue and cell culture applications.

OmniPur® Agarose PCR Plus features average gel strength, standard melting and gelling ranges, and is specifically designed to prevent smearing or high flurorescence backgrounds. Plus, this low electroendosmosis (EEO) agarose offers high electrophoretic mobility for shorter electrophoretic runs.

For a complete listing of OmniPur® grade reagents, visit www.merckbiosciences.com/OmniPur

Molecular Biology Reagents

Description	Size/Quantity	Catalogue No.
10 mM dNTP Mix	0.2 mL	71004-3
HEPES, Free Acid, Molecular Biology Grade	25 g 250 g	391340
Tris Base, Molecular Biology Grade	500 g 2.5 kg	648310
Tris Buffer, 1.0 M, pH 8.0, Molecular Biology Grade	100 mL	648314
Tris Buffer, 100 mM, pH 7.4, Molecular Biology Grade	100 mL	648315
TAE Buffer, 10X, Molecular Biology Grade	1 L	574797
TE Buffer, 100X, Molecular Biology Grade	1 L	574793
TBE Buffer, 10X, Molecular Biology Grade	1 L	574795

Molecular Biology Grade Reagents

Description	Size/Quantity	Catalogue No.
OmniPur® Agarose	100 g 500 g	2120 2125
EDTA, 0.5 M, pH 8.0, Molecular Biology Grade, DEPC-Treated	100 mL	324506
EDTA, Disodium Salt, Dihydrate, Molecular Biology Grade	100 g 1 kg	324503
Ethidium Bromide Adsorber	1 ea	331569
Formaldehyde, Molecular Biology Grade	250 mL	344198
X-Gal Solution	3 mL	71077-3
Sodium Chloride Tablets	1 ea (10 tablets)	567442

Molecular Biology Enzymes

Description	Size/Quantity	Catalogue No.
DNase I, RNase free	1,000 U	69182-3
DNase I, ds Qualified	50 U	69164-3
Phosphatase, Alkaline, Calf Intestine, Molecular Biology Grade	1,000 U	524576
Proteinase K Solution, 600 mAU/mL	2 mL 10 mL	71049-3 71049-4
Proteinase K, Lyophilized	100 mg 500 mg	70663-4 70663-5
RNase A, Protease-Free, Highly Purified, Bovine Pancreas	10 KU 50 KU	556746
RNase A Solution	1 mL	70856-3
T4 DNA Ligase	100 U 500 U	69839-3 69839-4
T4 Polynucleotide Kinase	250 U	69248-3

05 Related Products

Genome-scale Sample Preparation for Next Generation Sequencing

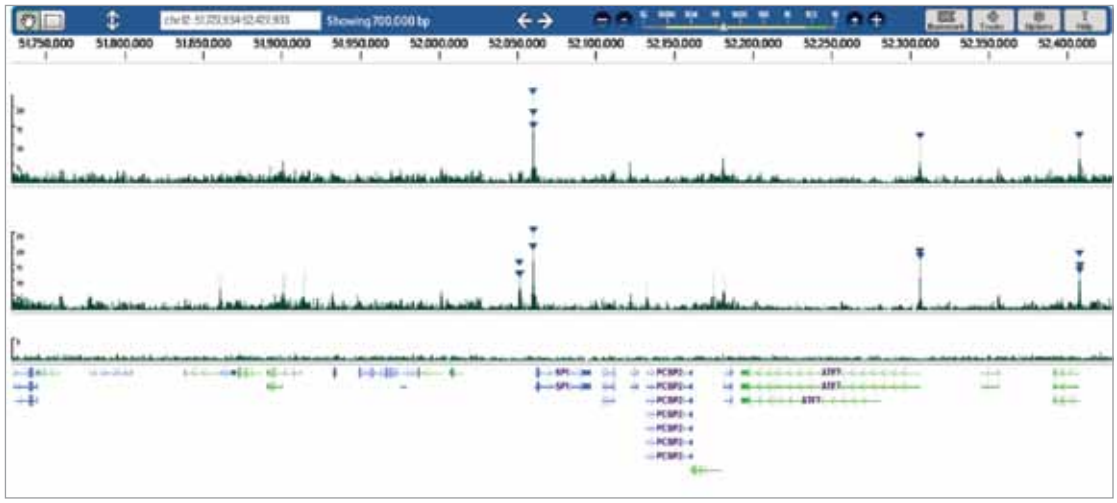
Simplifying genome-scale sample preparation for Next Generation Sequencing:

Magna ChIP-Seq™ Chromatin Immunoprecipitation and Next Generation Sequencing Library Construction Kit

Genome-wide mapping of DNA-protein interactions and epigenetic marks using chromatin immunoprecipitation combined with next generation sequencing (ChIP-Seq) is indispensable for many gene regulation studies. The Magna ChIP-Seq™ Kit simplifies this technique, enabling the performance of ChIP-Seq by virtually any laboratory.

Magna ChIP-Seq™ Advantages

- Reliable ChIP-Seq library construction from as little as 1 ng of purified ChIP DNA
- Magna ChIP™ Protein A+G bead blend is compatible with antibodies from virtually any species or class
- Flexible format allows construction of single end, paired end, or barcoded libraries
- Sufficient reagents for up to 10 next generation sequencing library constructions
- Expert support from our highly trained technical support scientists
- Quality-controlled, validated enzymes and buffers in convenient master mix streamline library construction
- Includes validated positive and negative control antibodies and a control primer set



Effective ChIP and reliable Next Gen Sequencing library construction from limited amounts of DNA. Sequencing libraries were constructed using the Magna ChIP-Seq™ Kit (Catalogue No. 17-1010) and the ChIPAb+™ Sp1 antibody/primer set (Catalogue No. 17-601). Libraries were constructed using 1 ng, 10 ng or an input chromatin sample and sequenced using an Illumina Genome Analyzer. Peak analysis (derived using quantitative enrichment of sequence tags (QuEST)) of the Sp1 locus from confidently mapped reads browsed with DNAnexus™ software shows Sp1 binding (triangles) occurs near expected Sp1 binding sites.

Fast and easy sample concentration and buffer exchange

Amicon® Ultra Centrifugal Filters

Amicon® Ultra Centrifugal filters provide fast sample processing and promote high sample recoveries, even in dilute samples, through ultrafiltration. The unique features of the Amicon® Ultra centrifugal filters give you the fastest, most efficient concentration for sensitive downstream applications.

Amicon® Ultra Centrifugal Filter Advantages: Maximize Concentration with Highest Protein Recovery

True Dead Stop

- Avoids spinning to dryness
- Provides a predictable concentration factor
- No need to calibrate for several samples to run parallel

Reverse Spin Recovery

- Reverse spin devices enable you to maximize protein recovery without introducing pipetting errors
- Low binding membrane and polypropylene housing for >90% sample recovery

Fast and Efficient Concentration Without Compromise

Vertical membranes

- Aligned with filtrate rather than perpendicular for less clogging, less waste and faster filtration
- Ultra-fast sample processing achieving concentration in as little as 10 minutes
- 25- to 80- fold concentration in a single step

Broad Chemical Compatibility

- Heat-sealed membrane eliminates adhesives and downstream extractables
- Large spectrum of compatibility
- Compatible with pH 1 to 9

Reliable Samples

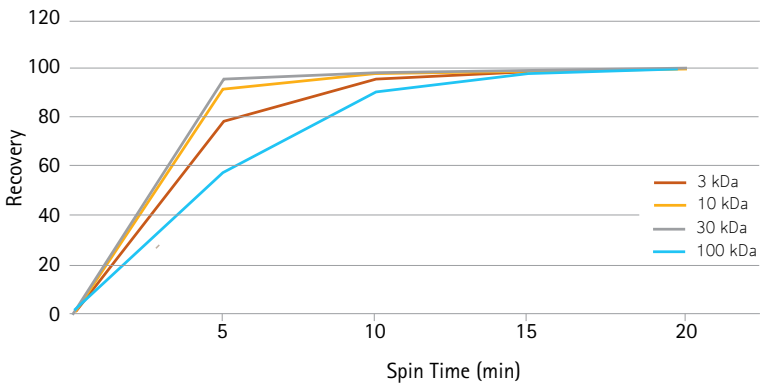
- Spin precious samples with confidence in one robust, sleek unit that prevents leakage

Amicon® Ultra-0.5

Amicon® Ultra-2

Amicon® Ultra-4

Amicon® Ultra-15



Amicon® Ultra 4 mL Filters – Fast Spin Times with Excellent Recovery.
Four different proteins (3 kDa Cytochrome C, 10 kDa Cytochrome C, BSA, and IgG) were tested on the Amicon® Ultra-4mL Filters for percent recovery and spin time. The data show that greater than 95% of all protein was recovered in 15 minutes or less.

Amicon® Ultra filters are available in various sizes and molecular weight cut offs (MWCO) to match your starting volume, molecular weight of protein or nucleic acid being concentrated, final volume and concentration factor.
Use the online Amicon® selector tool to choose the perfect filter and view protocols: www.millipore.com/AmiconSelect

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