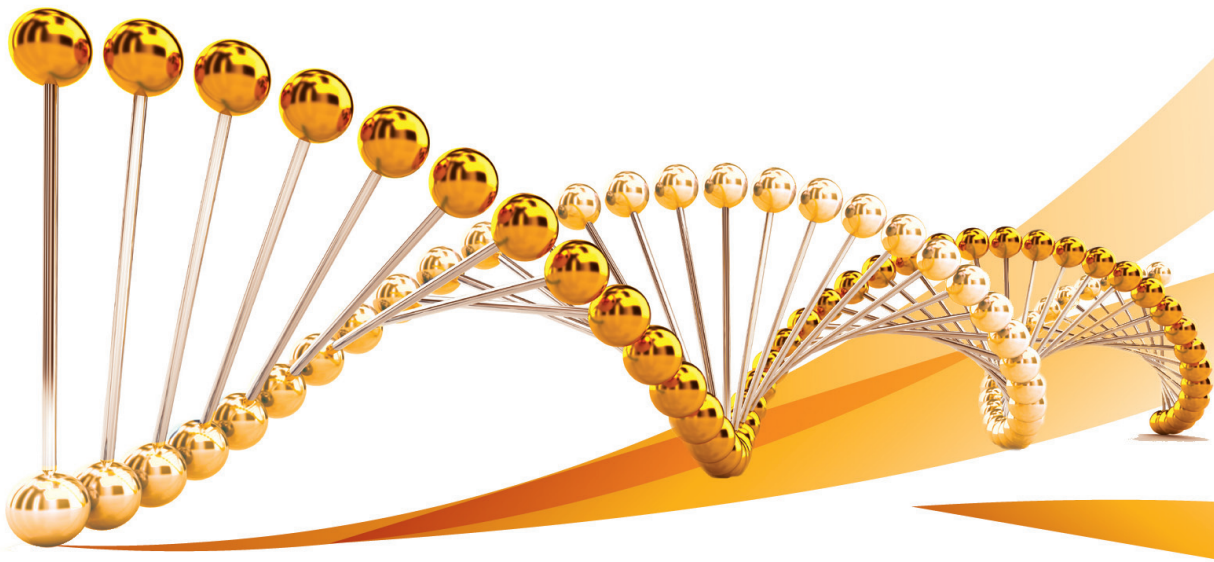
The Complete Molecular Biology Toolkit.
Expert workflow solutions from DNA cloning to protein expression.



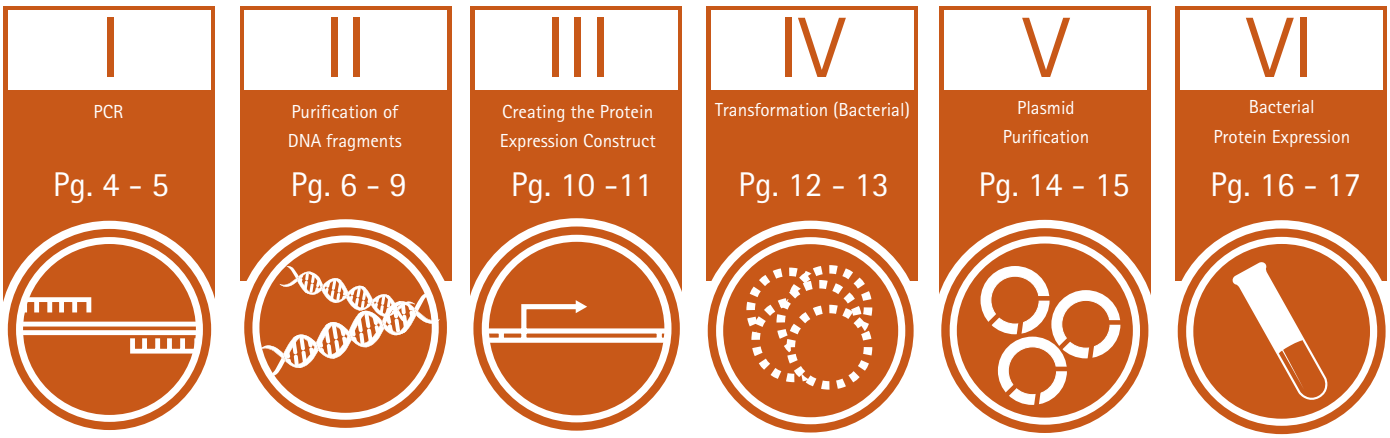
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Everything you need to clone DNA targets and purify native recombinant proteins.

Make discoveries faster by using the latest recombinant DNA tools that are specifically designed to meet your scientific and technical goals.

Whether you are expressing and purifying large amounts of recombinant protein to find links between structure and function, or whether you need to clone and express a genetically altered gene to examine its impact on a biological process, you need a complete set of molecular biology tools that work together.

EMD Millipore is your source for reagents, kits, cells, and tools for every step of the molecular biology and protein workflow. Partner with leading scientists on our technical support and research teams, and watch your research flourish.



I. PCR

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

EMD Millipore's molecular biologists work to develop the newest, best performing polymerases for customer use, optimizing conditions, buffer compositions, and cycling parameters to save you valuable time and resources.

Choose the Appropriate Thermostable Polymerase for PCR Applications:

Enzyme	KOD DNA Polymerase	KOD Hot Start DNA Polymerase	KOD XL DNA Polymerase	KOD Xtreme Hot Start DNA Polymerase
PCR Product Size (kb)	<6	<21	<30	<40
Applications	Cloning, cDNA amplification	Cloning, cDNA amplification	Crude samples, multiplex, incorporation of derivatized dNTPs	Crude samples, long targets, difficult and GC-rich targets

- KOD-XL DNA polymerase amplification results in a mixture of blunt and 3'-dA products while other KOD DNA polymerases generate blunt end products.
- NovaTaq™ is also available for routine PCR.

KOD DNA Polymerase is a Highly Efficient Proof Reading Enzyme

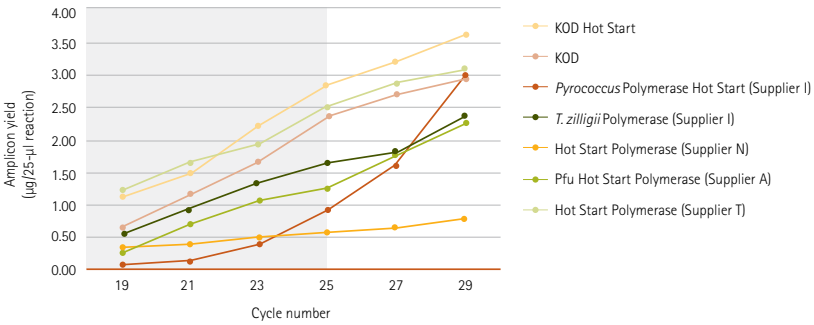
Enzyme	KOD DNA Polymerase	Pfu DNA Polymerase	Taq DNA Polymerase
Species	Thermococcus kodakaraensis	Pyrococcus furiosus	Thermus aquaticus YT-1
Fidelity† (mutation frequency)	0.0035	0.0039	0.013
Elongation rate (bases/second)	106-138	25	61
Processivity (nucleotide bases)	>300	<20	not determined

†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.

KOD Hot Start DNA Polymerase

This enzyme provides the highest accuracy, yield and processivity of our proofreading DNA polymerases. High processivity provides more product, enabling quick, reliable gene amplification. KOD Hot Start polymerase amplifies genomic DNA up to 12 kbp and plasmid DNA up to 21 kbp, including GC-rich regions. The polymerase is also available as mastermix for high throughput and standardization.

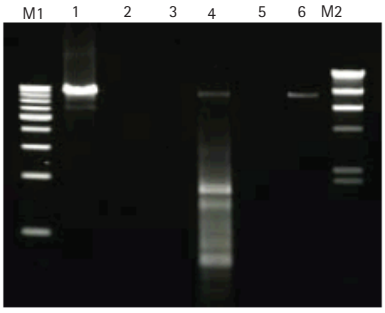
	Cycle Profile A	Cycle Profile B	Cycle Profile C	Cycle Profile D
Initial denaturation	98°C 30 s	94°C 2 min	95°C 2 min	95°C 2 min
	98°C 10 s	94°C 15 s	95°C 20 s	95°C 20 s
29 cycles	55°C 20 s	52°C 20 s	55°C 20 s	55°C 10 s
	72°C 30 s	68°C 60 s	72°C 30 s	70°C 15 s
Final extension	72°C 5 min	68°C 5 min	72°C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



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 out-performed the competition.

KOD Xtreme™ Hot Start DNA Polymerase

Use this optimized PCR system to reliably amplify long or challenging DNA templates (up to 90% GC content). Ideal for amplifying crude samples with minimal sample preparation, this kit translates into less reagents and less time spent on PCR.



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 λHindIII 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.

OmniPur® Agarose PCR Plus

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.emdbiosciences.com/OmniPur

ORDERING INFORMATION FOR KEY PRODUCTS
available at emdbiosciences.com

Description	Catalogue No.
KOD Hot Start DNA Polymerase	71086
KOD Hot Start Master Mix	71842
KOD Xtreme Hot Start DNA Polymerase	71975
KOD XL DNA Polymerase	71087
NovaTaq Hot Start DNA Polymerase	71091
OmniPur Agarose PCR Plus	2010
One Step RT-PCR Master Mix Kit	71978

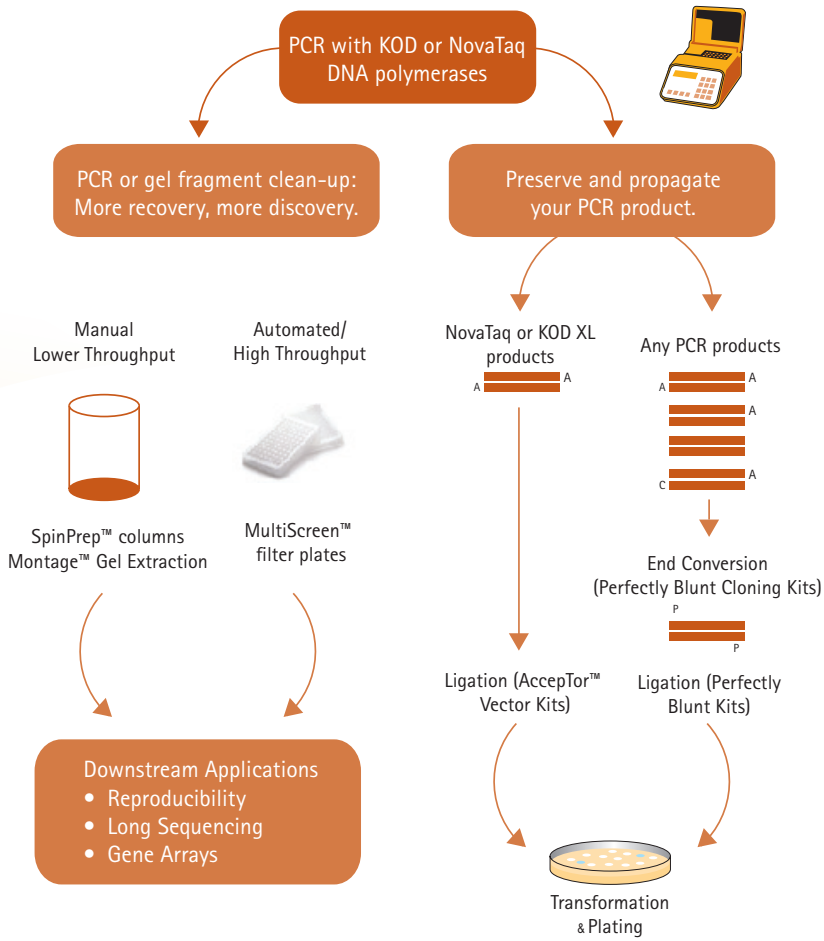
To place your order or for complete product information visit www.emdbiosciences.com

II. Purification of DNA Fragments and Subcloning

Downstream manipulations of PCR products, such as restriction digests, ligation, sequencing or hybridization, often require purification of the amplified DNA. Alternatively, PCR products can be directly ligated in plasmids, transformed and propagated in bacteria. Use schematic illustration below to help plan your workflow.

Whether you choose our PCR purification tools and gel extraction kits to obtain DNA fragments with the right purity and concentration for the next step, or whether you opt for one of our easy subcloning kits to preserve your product, you can expect superior recovery, accuracy, and efficiency.

Workflow Guide for Sample Preparation of PCR Products

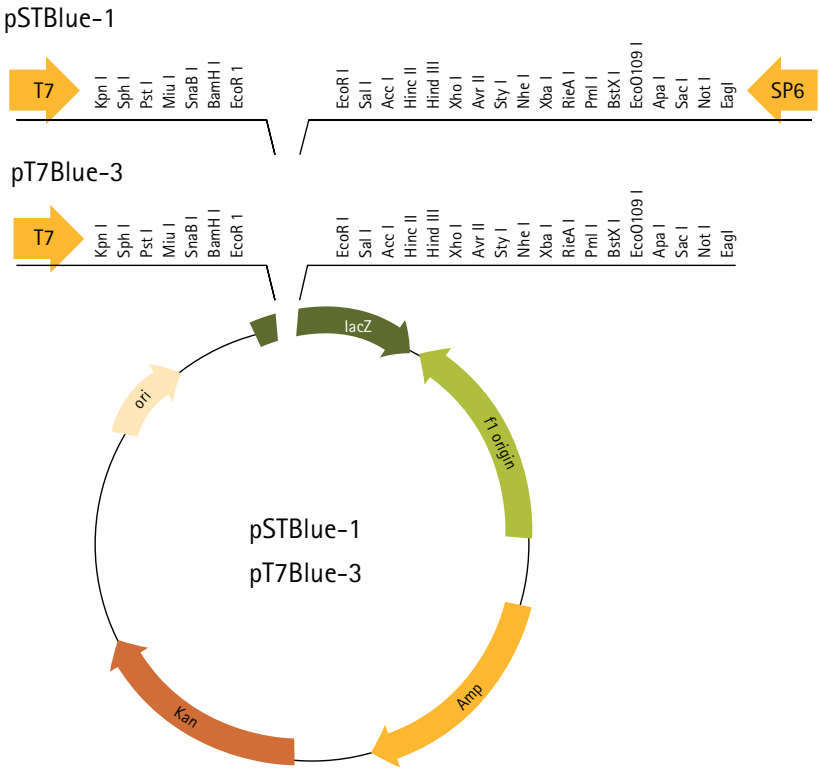


AccepTor™ Vector Kits

Easily and quickly clone PCR products without restriction digests or special primers. Use AccepTor vector kits to clone PCR products with single 3´-dA overhangs, which are generated by DNA polymerases such as KOD XL and Taq polymerases. The ready-to-ligate AccepTor Vector contains single 3´-dU ends. Simply mix the vector with your PCR product, incubate with Clonables™ 2X Ligation Premix, and transform into NovaBlue Singles™ Competent Cells—the entire process can be completed in just 40 minutes.

Perfectly Blunt Cloning Kits

Efficiently clone DNA with any type of end in less than an hour, with no restriction digests or special primers. Perfectly Blunt Cloning Kits are a convenient platform for subcloning DNA sequences amplified with KOD polymerase, which generates blunt ends. Alternatively, use the kit's End Conversion Mix to to produce blunt, phosphorylated ends, which are compatible with the linearized, dephosphorylated blunt vector. This approach enables simple cloning after amplification with high-fidelity proof reading enzymes, which decreases the probability of mutations in the target sequence. In addition, blunt cloning can be more efficient than T-cloning. These kits are also perfect for cloning restriction fragments, cDNA, or sheared DNA.



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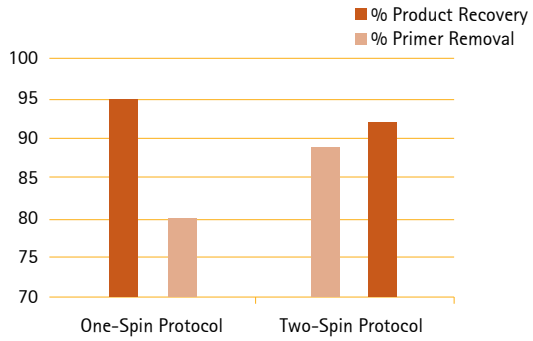
Description	Catalogue No.
pSTBlue-1 AccepTor Vector Kit	70595
pETBlue-1 AccepTor Vector Kit	70598
pT7Blue-3 Perfectly Blunt Cloning Kit	70182
pT7Blue-2 Perfectly Blunt Cloning Kit	70190
pT7Blue Perfectly Blunt Cloning Kit	70189
pETBlue-1 Perfectly Blunt Cloning Kit	70634
pETBlue-2 Perfectly Blunt Cloning Kit	70636
pSTBlue-1 Perfectly Blunt Cloning Kit	70191
Clonables 2X Ligation Premix	70573

To place your order or for complete product information visit www.emdbiosciences.com

Amicon® Ultra 0.5 mL Centrifugal Filters, MWCO 30,000

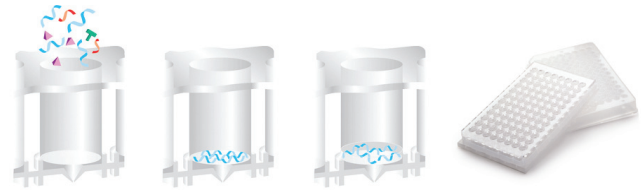
Centrifuging PCR reactions through an ultrafiltration membrane is a fast, easy way to separate DNA amplicons from other reaction components. With its engineered dead stop and vertical membrane design, the industry-leading Amicon Ultra filter provides the best yields with the fastest spin times, and enables simultaneous concentration and purification of DNA fragments.



Additional wash optimizes primer removal while maintaining yield when separating a PCR product from primers with Amicon Ultra 0.5 mL filters. 100 µL of PCR product in Tris-EDTA (TE) buffer was spiked with fluorescently labeled primers, and spun for 12 min. To half of the devices, an additional 500 µL of TE was added and spun again. After a final reverse spin, the amount of primers and PCR product (using fluorescence) was determined in each retentate and filtrate. The chart shows percent PCR product recovery and percent primer removal using either a single elution (left) or additional wash (right).

MultiScreen PCR Filter Plates

PCR filter plates are fast, automatable solutions for high-throughput PCR purification. Available in 96- and 384-well formats as well as micro 96-well format for small volumes, MultiScreen PCR plates provide high purity and recovery with a much shorter protocol and fewer handling steps than other methods. The plates use a size-exclusion membrane and vacuum filtration to provide a one-step protocol for excellent results. No centrifugation or precipitation steps are required.



1. 2. 3.

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

1. Load PCR reactions.
2. Filter with EMD 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.

Proven workflow successes with below automated platforms:

- Biomek® FX Nucleic Acid Preparation System
- Packard MultiPROBE® II EX Liquid Handling System
- Beckman Multimek96 Pipettor

SpinPrep™ PCR Clean-Up Kit

Combine simplicity, speed, range and sensitivity when you use this kit to clean up and recover PCR-amplified DNA. The 3-step, 10-minute spin column procedure involves adsorption of DNA to silica membrane activated with binding buffer. After a wash step, DNA is eluted using a low-salt buffer. Effectively remove DNA polymerases, dNTPs, salts and primers from the PCR reaction mix to avoid interference with downstream applications such as cloning, sequencing or labeling. Purify PCR products from 100 bp to > 12 kbp with up to 90% recovery.

Spinprep PCR Clean-Up Kit

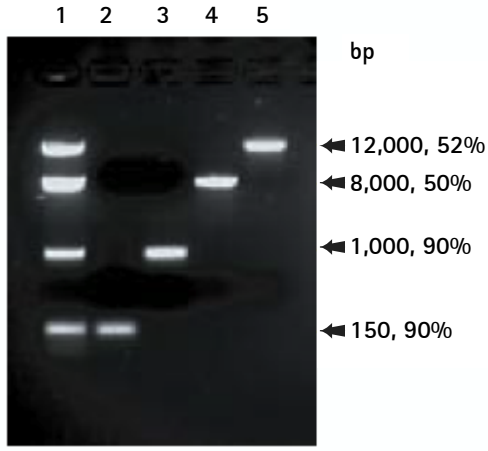
Column binding capacity:	up to 6 µg
PCR sample volume:	100 µL/rxn
Typical recovery:	60-90%
Size range:	100 bp to > 12,000 bp
Time required:	< 10 min

SpinPrep Gel DNA Kit

Quickly and efficiently extract DNA fragments from 150 bp to >12,000 bp from agarose gels without organic extraction or alcohol precipitation. The procedure uses GelMelt™ Solution to dissolve the gel slice, followed by adsorption of the DNA to a silica membrane in a spin column format. After a wash step, the purified DNA is eluted in low-salt buffer.

SpinPrep Gel DNA Kit

Column binding capacity:	up to 20 µg
Gel slice mass:	150 mg/rxn
Typical recovery:	50-90%
Size range:	150 bp to > 12,000 bp
Time required:	< 30 min



Gel analysis and quantification of DNA fragments isolated with the SpinPrep Gel DNA Kit

Known amounts (2 µg) of four DNA fragments of the indicated sizes 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 and standard protocol. Recoveries shown as percentages above were determined by absorbance at 260 nm. Samples (250 ng) of each recovered band were analyzed by agarose gel electrophoresis. Lane 1 contained a mixture of the starting DNAs.

ORDERING INFORMATION FOR KEY PRODUCTS
available at emdbiosciences.com

Description	Catalogue No.
SpinPrep PCR Clean-Up Kit	70976
SpinPrep Gel DNA Kit	70852

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ORDERING INFORMATION FOR KEY PRODUCTS
available at millipore.com

MultiScreen PCR_{96} Filter Plates	MSNU3050
Amicon Ultra 0.5 mL Centrifugal Filters	UFC503096
Montage® Gel Extraction Kit	LSKGEL050

To place your order or for complete product information visit www.millipore.com

III. Creating the Protein Expression Construct

Efficiently express your target protein by cloning your gene into one of our panel of carefully designed expression plasmids. Offering a choice of bacterial, mammalian or insect cell systems, EMD Millipore's vast portfolio of expression vectors enables you to choose the perfect combination of promoters, epitope tags, antibiotic resistance, and host compatibility. With our vectors and cloning kits, you can generate high performance constructs in as little as 40 minutes.

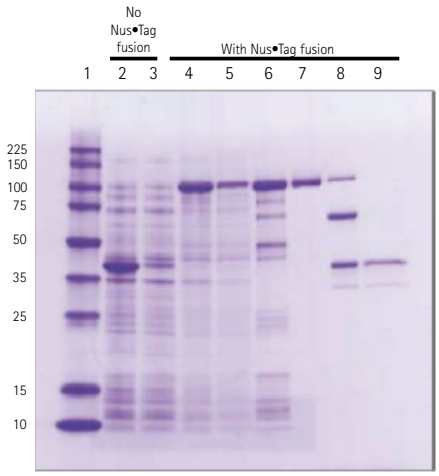
Featured Products

pET *E. coli* T7 Expression Vectors

The pET System is the most powerful system for the cloning and expression of recombinant proteins in *E. coli*. Driven by the strong bacteriophage T7 promoter and translation signals, Novagen's® pET System has been used to express thousands of different proteins in host cells expressing T7 polymerase. The pET System has continuously expanded to offer new technologies and options for expression, and includes a large collection of pET vector types, different host strains and companion products designed for efficient detection and purification of target proteins.

- pET-31b(+) for high yield bioproduction of peptides and small proteins
- pET-32a-c for production of soluble, active target proteins in *E. coli*
- pET-39b and 40b using Dsb tags for export and periplasmic folding of target proteins
- pET-41a-c and 42a-c with popular GST fusion tags for enhanced production and solubility
- pET-43.1a-c designed for cloning and high-level expression of polypeptide sequences fused with the 495 aa NusA (Nus•Tag™) protein
- pET-44a-c with Nus•Tag sequence plus N- and C-terminal His•Tag sequences
- pET-45b(+) with amino-terminal His•Tag™ sequence and minimal extraneous sequences
- pET-46 Ek/LIC prepared vector for ligation-independent cloning, with amino-terminal His•Tag sequence
- pET-47b(+) through pET-50b(+) with HRV 3C Protease cleavage site for efficient fusion tag removal

Nus•Tag™ fusion increases the solubility of recombinant annexin A1.



Lane	Sample
1	Perfect Protein™ markers 10-225 kDa
2	Total cell protein (TCP)
3	Soluble fraction
4	Total cell protein (TCP)
5	Soluble fraction
6	Ni^{2+} His•Bind® Fractogel eluate pool
7	Strep•Tactin® eluate pool
8	Thrombin digest
9	Post-thrombin His•Bind flowthrough

pENTR™ vectors containing the annexin A1 target gene were generated using the Gateway Nova pET-53-DEST™ vector (lanes 2-3, pET-His•Tag®/annexin-A1/Strep•Tag® II) and Gateway Nova pET-57-DEST vector (lanes 4-5, pET-His•Tag/Nus•Tag/thrombin/annexin-A1/Strep•Tag II).

Soluble annexin A1 was purified on sequential His•Bind and Strep•Tactin columns (lanes 6-7). The N-terminal fusion tags were removed by thrombin cleavage and a subtractive His•Bind column (lanes 8-9).

Proteinase K

Use Proteinase K to stop enzymatic reactions, including endonucleolytic cleavage and polynucleotide addition, and efficiently remove proteins from nucleic acid preparations. Proteinase K is a highly active serine protease that exhibits broad cleavage specificity on native and denatured proteins and is widely used in the purification of DNA and RNA. Its activity is increased in the presence of denaturants such as SDS (1%) and elevated temperature (50-60°C). The enzyme is available lyophilized or as a ready-to-use concentrated stock solution (600 mAU/mL). Novagen Proteinase K products are free of detectable DNase and RNase.

ORDERING INFORMATION FOR KEY PRODUCTS
available at emdbiosciences.com

Description	Catalogue No.
pET-31b(+) DNA	69952
pET-32a(+) DNA	69015
pET-32b(+) DNA	69016
pET-32c(+) DNA	69017
pET-39b(+) DNA	70090
pET-40b(+) DNA	70091
pET-41a(+) DNA	70556
pET-41b(+) DNA	70557
pET-41c(+) DNA	70558
pET-42a(+) DNA	70561
pET-42b(+) DNA	70562
pET-42c(+) DNA	70563
pET-43.1a(+) DNA	70939
pET-43.1b(+) DNA	70940
pET-43.1c(+) DNA	70941
pET-44a(+) DNA	71122
pET-44b(+) DNA	71123
pET-44c(+) DNA	71124
pET-45b(+) DNA	71327
pET-46 Ek/LIC Vector Kit	71335
pET-47b(+) DNA	71461
pET-48b(+) DNA	71462
pET-49b(+) DNA	71463
pET-50b(+) DNA	71464
Proteinase K, lyophilized	70663
Proteinase K Solution, 600 mAU/mL	71049

To place your order or for complete product information visit www.emdbiosciences.com

IV. Transformation (Bacterial)

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. EMD Millipore offers a range of strains and formats to suit every cloning project. 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 protein and molecular biology research, we offer robust performance you can count on.

Featured Products

NovaBlue Competent Cells

For routine initial cloning, time-tested NovaBlue Competent Cells are ideal. NovaBlue is a K-12 derivative that offers high transformation efficiency, facilitates plasmid stability, and allows blue/white screening with appropriate plasmids.

Concerned about phage resistance? NovaBlue T1R is resistant to T1 and T5 phage. And when selecting for pEXPR constructs using Gateway® Nova pDEST™ vectors or when cloning into pETcoco™ vectors, NovaF- is the right tool for the job.

NovaBlue is a K-12 derivative that offers guaranteed transformation efficiency of >1.5 x 10^8 cfu/µg, facilitates plasmid stability, and allows blue/white screening with appropriate plasmids.

NovaBlue Singles Competent Cells are designed for ultimate convenience and reliability in plasmid transformation. Cells are provided in 50 µL volumes to eliminate the need to aliquot, freeze/thaw, or waste partially used vials. This saves time, money, and ensures reliable cell performance.

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

*Measured as cfu/µg test plasmid

NovaBlue Genotype:

*endA1 hsdR17(r_{K12}-m_{K12}+) supE44 thi-1 recA1 gyrA96 relA1 lac F'[proA$^+$B$^+$ lacI^{q}ZΔM15::*Tn*10]* (Tet^R)

Veggie NovaBlue Singles Competent Cells

For cloning applications in which absence of animal-derived components is essential, use Veggie NovaBlue Singles Competent Cells. Like all NovaBlue competent cells, Veggie NovaBlue is a K-12 strain featuring high transformation efficiency, blue/white screening capability (with appropriate plasmids), and mutations in recA and endA, which result in excellent yields of high-quality plasmid DNA. Veggie NovaBlue Singles Competent Cells are supplied with an animal-free prepared SOC medium.

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Description	Catalogue No.
NovaBlue Singles Competent Cells	70181
Veggie NovaBlue Singles Competent Cells	71251
HT96 NovaBlue Competent Cells	71011
NovaBlue T1^R Singles Competent Cells	71318

To place your order or for complete product information visit www.emdbiosciences.com

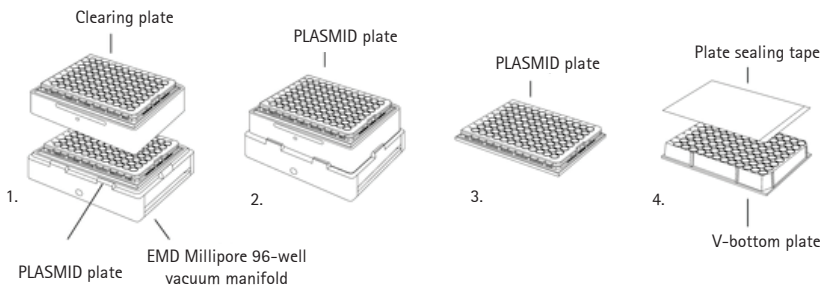
V. Plasmid Purification

Small-scale plasmid purifications ("minipreps") are required for identifying bacterial clones that contain engineered constructs with the correct insert, correct sequence, and optimal growth properties. High quality miniprep DNA can also be used directly for high throughput sequencing, library construction, RNAi, or other cell-based screens. With over 50 years of experience in membrane filtration, EMD Millipore offers filtration-based plasmid purification tools to increase miniprep throughput without compromising DNA quality, enabling both faster decisions and reproducible data.

Featured Products

Montage Plasmid Miniprep$_{96}$ Kit

The Montage Plasmid Miniprep$_{96}$ Kit is a automation-compatible, easy-to-use kit for high throughput, high-purity plasmid or bacterial artificial chromosome (BAC) minipreps. Using a patented separation technology, the Montage Kit follows a simple protocol that eliminates lengthy bind/elute methods and centrifugation steps to yield clean and reproducible DNA in 50% less time than traditional methods. Following lysis, only three short filtration steps are required to prepare 96 DNA samples from each plate.



Perform high throughput minipreps using the Montage Plasmid Miniprep$_{HTS}$ Kits:

1. Following bacterial lysis, transfer lysates from deep well culture block into clearing plate. Clear lysates into Plasmid filter plate using vacuum.
2. Move Plasmid plate to top of manifold and apply vacuum for 5 to 7 minutes to concentrate sample. Plasmid DNA is retained on the membrane surface while contaminants are filtered to waste. Add wash solution and filter for 3 to 5 minutes.
3. Add resuspension buffer and collect purified sample by aspiration.
4. Transfer samples to V-bottom plate for use or storage.

High Yields From Montage Plasmid Miniprep$_{96}$ Kits*

Host strain	Plasmid	Yield (µg) per well
XL 1-blue	pUC19	2.1 – 6.1
	pLH2**	3.5 – 9.2
JM109	pUC19	2.4 – 6.4
	pLH2**	3.5 – 9.1

*Results will vary depending on host strain, plasmid, and protocol conditions.

**pLH2 was derived from pUC19. The 2.0 Kb fragment of *Hin*d III digested I phage was cloned into the *Hin*d III site of pUC19, resulting in a 4.7 Kb plasmid.

ORDERING INFORMATION FOR KEY PRODUCTS
available at millipore.com

Description	Catalogue No.
Montage Plasmid Miniprep$_{96}$ Kit	LSKP09604
Montage 384-well Plasmid Miniprep Clearing Plates	LSKP38450
MultiScreen$_{HTS}$ Vacuum Manifold	MSVMHTS00

To place your order or for complete product information visit www.millipore.com

VI. Bacterial Protein Expression

When expressing recombinant proteins in *E. coli,* the goals are to obtain high yields of full-length, soluble protein. Whether you are producing protein for enzymatic assays, generating antigen for antibody production, assaying protein-protein interactions, or determining three-dimensional structure – you need high-quality protein, fast. EMD Millipore's portfolio of bacterial strains for protein expression includes the best all-purpose strains and several specialty strains for difficult-to-express proteins, all backed by unwavering technical support to increase your chances of success. For ultimate convenience and reliability in plasmid transformation, Singles Competent Cells are provided in 50 µL volumes to eliminate the need to aliquot, freeze/thaw, or waste partially used vials. This saves time, money, and ensures reliable cell performance.

Featured Products

BL21(DE3) Singles Competent Cells

BL21 has been the gold standard for protein expression since it was first introduced in 1990. Deficient in Lon and OmpT proteases, BL21 and its derivatives are ideal for many applications. T7 expression strains are lysogens of bacteriophage λDE3, as indicated by the (DE3), and express T7 RNA polymerase. Such strains are suitable for production of protein from target genes cloned in appropriate T7 expression vectors.

BL21(DE3)pLysS Singles Competent Cells

The pLysS designation is given to strains carrying a plasmid that encodes T7 lysozyme, a natural inhibitor of T7 RNA polymerase. These strains are used to suppress basal expression of T7 RNA polymerase prior to induction and thus stabilize pET, pCDF, pRSF, pACYCDuet™, pCOLADuet™, and Gateway® Nova pDEST™ recombinants encoding target proteins that affect cell growth and viability.

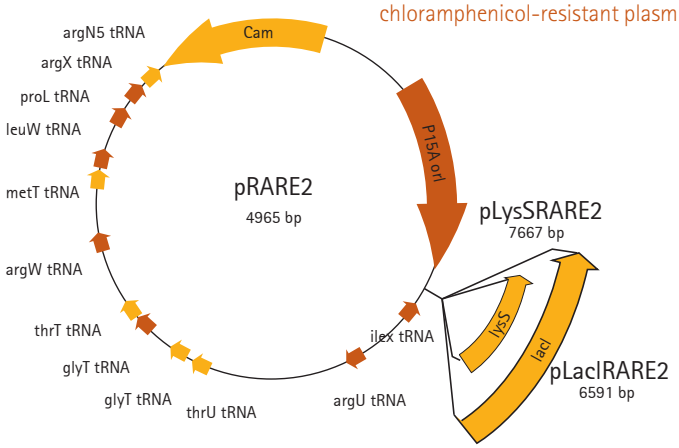
Origami™ 2(DE3) Singles Competent Cells

Recombinant proteins that depend on disulfide bond formation for proper folding may be difficult to express in bacterial cytoplasm, which is typically a reducing environment. Origami 2 and Origami B strains have mutations in glutathione reductase (gor) and thioredoxin reductase (trxB), facilitating proper disulfide bond formation and increasing yields of folded, soluble protein.

Rosetta™ 2(DE3) Singles Competent Cells

Reduce protein truncation with Rosetta 2 host strains. These strains are BL21 derivatives that enhance the expression of eukaryotic proteins containing codons rarely used in *E. coli*. These strains supply tRNAs for 7 rare codons (AGA, AGG, AUA, CUA, GGA, CCC, and CGG) on a compatible chloramphenicol-resistant plasmid. The tRNA genes are driven by their native promoters.

Rosetta 2 strains express 7 rare codons on a chloramphenicol-resistant plasmid, pRARE2.



HMS174(DE3)pLysS Competent Cells

Are toxic recombinant proteins causing problems with bacterial growth? The HMS174 strain provides high transformation efficiency (>5 x 10^6 cfu/µg) and the recA mutation in a K-12 background. This mutation may stabilize certain target genes whose products may cause the loss of the DE3 prophage.

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Description	Catalogue No.
BL21(DE3) Singles Competent Cells	70235
BL21(DE3)pLysS Singles Competent Cells	70236
Veggie BL21(DE3) Singles Competent Cells	71252
Veggie BL21(DE3)pLysS Singles Competent Cells	71253
Origami 2(DE3) Singles Competent Cells	71408
Rosetta 2(DE3) Singles Competent Cells	71400
Rosetta 2 (DE3) pLysS Singles Competent Cells	71401
HMS174(DE3) Competent Cells	69453

To place your order or for complete product information visit www.emdbiosciences.com

VII. Transfection

Successful expression of recombinant proteins in mammalian or insect cells requires a transfection protocol that maximizes transfection efficiency, minimizes cytotoxicity, and results in the desired level of protein expression. EMD Millipore recognizes that there is not a one-size-fits-all solution to your transfection needs. We provide the breadth of transfection reagents necessary to give you the freedom to design the perfect experiment. Simplified protocols, including no media changes for most reagents and cell types, have been pre-optimized, requiring minimal assay development and allowing more time for research.

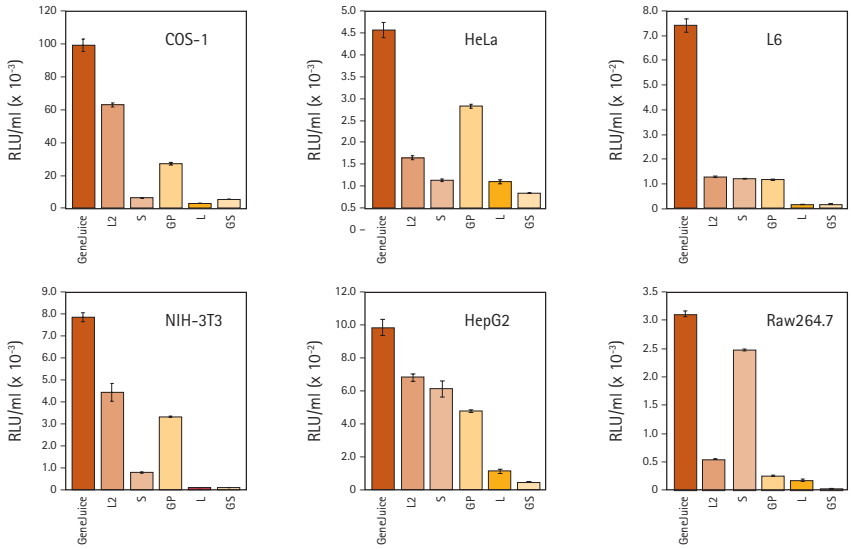
Featured Products

GeneJuice® Transfection Reagent

For high-efficiency transfection of a wide variety of mammalian cells, depend on GeneJuice Transfection reagent, a proprietary formulation optimized for maximal transfection efficiency, ease of use, and minimal cytotoxicity. A superior alternative to lipid-based reagents and other traditional techniques, GeneJuice Transfection Reagent is composed of a nontoxic cellular protein and a small amount of a novel polyamine. The unique chemistry provides several advantages over lipid-based transfection, including:

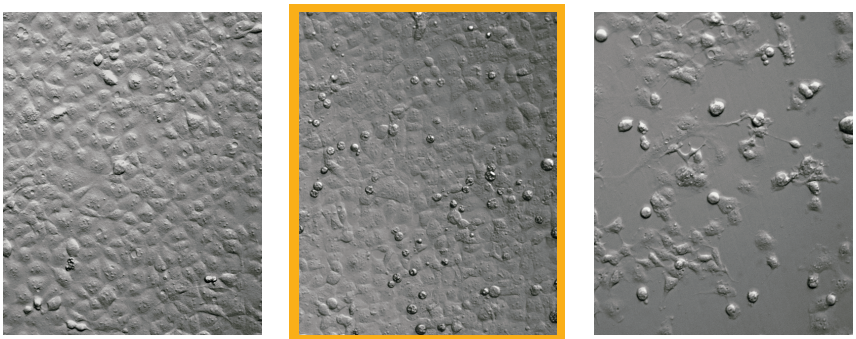
- Highly efficient DNA transfer for both stable and transient transfections
- Minimal cellular toxicity
- Compatibility with both serum-containing and serum-free media
- Simple protocol—no need for media changes
- Ideal for high throughput transfection in multi-well plate formats

GeneJuice Reagent Enables Greater Transfection Efficiency Versus Commonly Used Competitor Reagents



The indicated cell lines were plated at a density of 3×10^4 cells per well in 24-well plates the day prior to gene delivery. Transfections and media changes were performed according to the manufacturers' optimized protocols. For transfection, 0.5 µg of low endotoxin purified pTriEx™-4 Fluc plasmid DNA was complexed with the relevant reagent and introduced into each well. After 48 h, the cells were extracted with Reportasol™ Extraction Buffer and Fluc activity was assayed. Data are represented as relative light units per milliliter of extract (RLU/mL). All values reflect an average of four replicate cultures.

GeneJuice Reagent Is Less Cytotoxic Than Lipid-Based Transfection Reagents



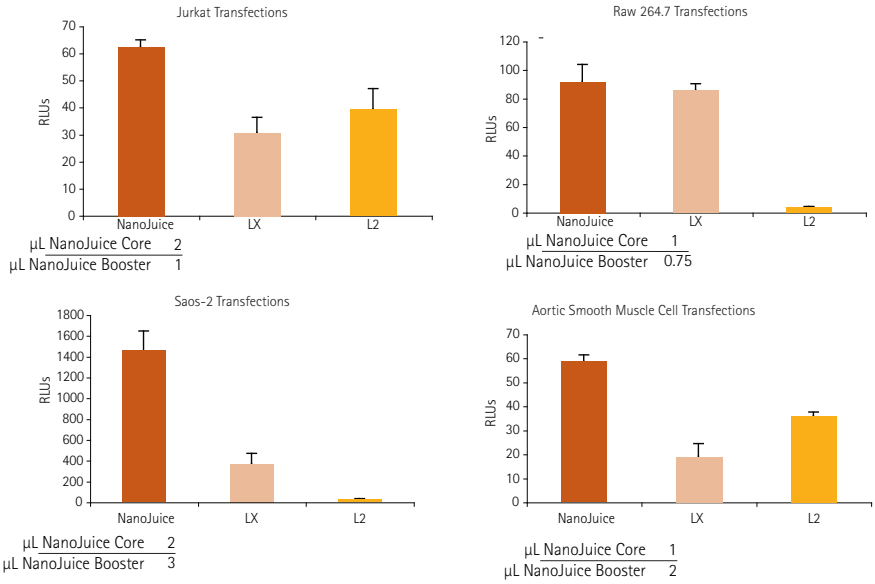
No transfection | **GeneJuice Reagent** | Reagent L2

Three replicate COS-7 cultures were left untreated, transfected with a popular cationic lipid-based transfection reagent, or transfected with GeneJuice Transfection Reagent according to recommended protocols. Cellular damage is visualized by rounding up and detachment from the plate surface. The photographs were taken 48 h post transfection.

NanoJuice® Transfection Kit

Efficiently transfect difficult-to-transfect mammalian cell types with NanoJuice Transfection Kit, which is comprised of two separate reagents developed to work synergistically. Using the cutting-edge nanotechnology of Priostar® dendrimers along with a polycationic liposomal formulation, the NanoJuice reagent mixture provides excellent transfection rates, low cytotoxicity, and the methodological flexibility needed for primary cells and other difficult-to-transfect cells.

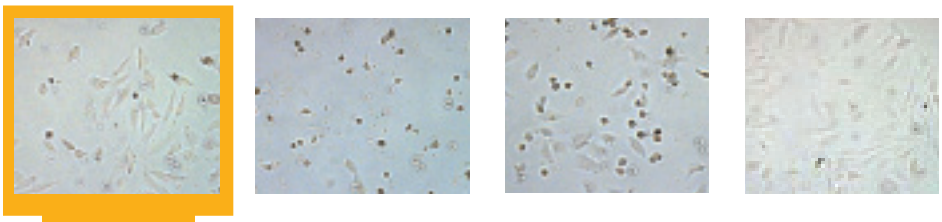
NanoJuice Transfection Kit Enables Greater Transfection Efficiency For Difficult Cell Types Versus Commonly Used Competitor Reagents



The indicated cell lines were plated in 24-well plates 18-24 h prior to transfection, such that cells were 80% confluent at time of transfection. Transfections were performed according to the manufacturers' optimized protocols. For transfection, 0.25 µg of low endotoxin purified pTriEx-6 Rluc plasmid DNA was complexed with the relevant reagent and introduced into each well.

After 24-48 h, the cells were extracted with Reportasol Extraction Buffer and Rluc activity was assayed. Data are represented as relative light units per well of 24-well plate (RLU/well). All values reflect an average of three replicate cultures.

NanoJuice Is Less Cytotoxic Than Competitor Reagents

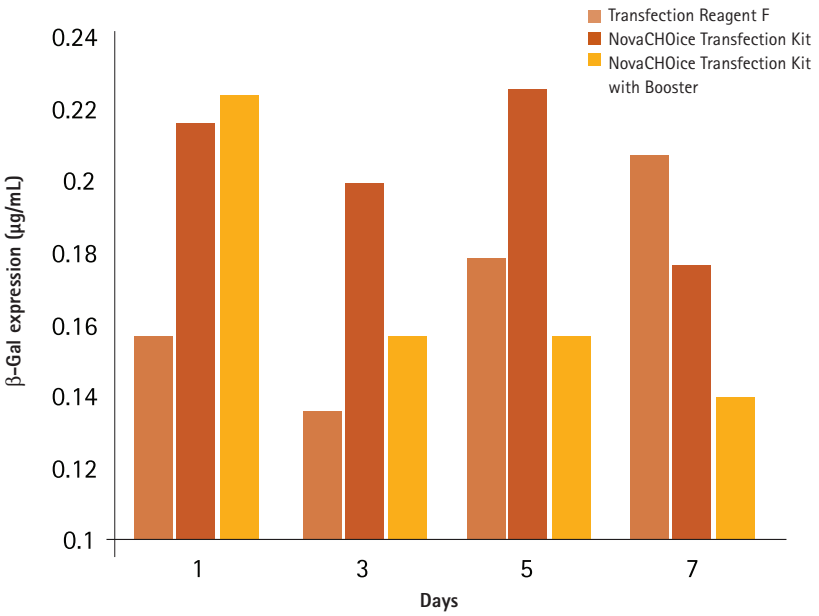


NanoJuice Transfection Kit | Reagent L2 | Reagent LX | No Transfection

Three replicate Saos-2 cultures were either left untreated, transfected with commonly used competitor's reagents, or transfected with NanoJuice Transfection Kit according to recommended protocols. Photographs were taken 48 h post-transfection.

NovaCHOice™ Transfection Kit

Chinese hamster ovary (CHO) cells are an excellent mammalian host system for transfection, recombinant protein expression and purification of authentically glycosylated and post-translationally folded proteins that resemble their natural counterparts. EMD Millipore's NovaCHOice Kit provides optimal transfection in this widely utilized cell line. Quality controlled against ATCC CHO-K1 cells, the NovaCHOice Kit is formulated to offer highly efficient DNA delivery with minimal cell death to maximize recombinant protein expression.



Detect protein expression earlier when you trasfect with the NovaCHOice Transfection Kit.

β-Galatosidase expression data in CHO-S cells at 1, 3, 5 and 7 days post transfection using the NovaCHOice Transfection Kit and a competing reagent according to manufacturer's protocol. Results show that higher protein expression was detected on Day 1 in cells trasfected with NovaCHOice Transfection Kit than in those transfected with the Transfection Reagent F.

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Description	Catalogue No.
GeneJuice Transfection Reagent	70967
NanoJuice Transfection Kit	71902
293-Free™ Transfection Reagent	72181
Insect GeneJuice Transfection Reagent	71259
ProteoJuice™ Protein Transfection Reagent	71281
RiboJuice™ siRNA Transfection Reagent	71115
NovaCHOice Transfection Kit	72622

To place your order or for complete product information visit www.emdbiosciences.com

VIII. Expansion of Cell Cultures for Protein Expression

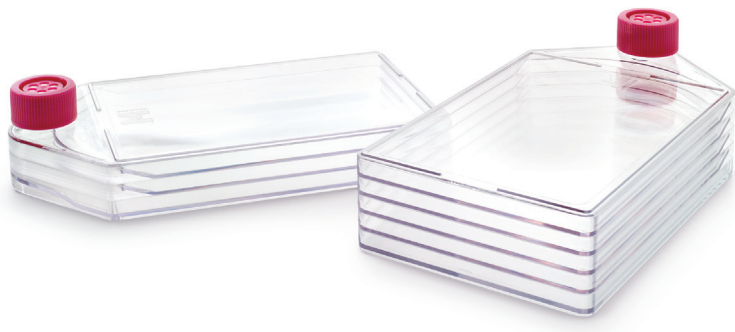
Growing large numbers of mammalian or insect cells for protein expression is often challenging because of limited incubator space, risk of contamination, extended bench time required for passaging cells, poor yields from culture flasks, and high cost of media and reagents.

EMD Millipore's Millicell® HY Multilayer Culture Flasks address these challenges. Together with our Calbiochem® cell-culture tested antibiotics, they make selecting and expanding transfected cell cultures easy and reproducible.

Featured Products

Millicell HY (High Yield) Cell Culture Flasks

Depend on Millicell HY cell culture flasks for uniform, consistent cell growth, which enables the amazingly high cell yields provided by these unique flasks. When choosing a multilayer cell culture flask to save space, time and – most importantly – grow healthy cells, trust in EMD Millipore to deliver a flask that outperforms the rest.



Millicell HY Culture Flask Feature:	How It Helps You:
Consistent, quality growth environment across all layers	Better cell proliferation and yields than other multilayer flasks
Unique, ergonomic design	Easy cell passaging and recovery
No leaking or spillover between layers	Reduced risk of contamination
Multilayer format	Saves you: • Incubator space • Time spent passaging cells • Cost of reagents

Flask	% of Theoretical yield*	Flasks required**	Volume of medium required	The Millicell Benefit
Millicell 3 Layer - 600 cm^2	97%	1	150 mL	• Less Media • Fewer Flasks • Save Incubator Space • Time Savings
Brand N T75-75 cm^2	100%	8	169 mL	
Brand N 3 Layer - 500 cm^2	79%	1.5	189 mL	
Millicell 5 Layer - 1000 cm^2	90.5%	n/a	300 mL	• Less Media • Healthier Cells • Cost Savings
Brand C 10 Layer - 1720 cm^2	55%	n/a	560 mL	

* Per unit surface area ** For same cell yield as Millicell HY flask

Antibiotics and Selection Agents

To select cells that have been successfully transfected with DNA carrying an antibiotic resistance marker, choose from our wide variety of high quality Calbiochem cell-culture tested antibiotics. Cited in thousands of peer-reviewed journals, Calbiochem antibiotics are manufactured in a controlled environment and perform with exceptional consistency to ensure the best results, lot after lot. Examples of Calbiochem antibiotics frequently cited in publications:

Antibiotic	Catalogue Number	# of Citations from 2000-2009
G-418 Sulfate, cell culture tested	345810	6200
Bafilomycin A1, *Streptomyces griseus*	196000	460
Hygromycin B, *Streptomyces* sp., cell culture tested	400052	2550

For more information, visit www.emdbiosciences.com/Antibiotics

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Description	Catalogue No.
G-418 Sulfate	345810
Hygromycin B	400052
Puromycin	540411

To place your order or for complete product information visit www.emdbiosciences.com

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Description	Catalogue No.
Millicell HY Culture Flask, 3 layer, T600, sterile	PFHYS0616
Millicell HY Culture Flask, 5 layer, T1000, sterile	PFHYS1008

To place your order or for complete product information visit www.millipore.com

IX. Cell Lysis and Protein Extraction

Obtaining maximal yields of soluble proteins from cells requires efficient cell lysis. However, overly harsh lysis conditions can denature or degrade the protein of interest. EMD Millipore has developed many reagents to enable gentle, efficient cell lysis.

Our BugBuster®, YeastBuster™, and CytoBuster™ Protein Extraction Reagents are innovative combinations of detergents and other ingredients that enable non-mechanical extraction of soluble proteins from bacteria, yeast, plant, mammalian, and insect cells. rLysozyme™ Solution increases the efficiency of bacterial lysis with BugBuster Reagent.

Addition of Benzonase® Nuclease specifically degrades contaminating DNA and RNA for the preparation of non-viscous, nucleic acid-free extracts ready for target protein purification. Lysonase™ Bioprocessing Reagent combines the functional activities of rLysozyme and Benzonase Nuclease in an optimized, ready-to-use reagent that significantly increases protein extraction efficiency and facilitates processing of protein extracts.

Protease Inhibitor Cocktails and Inhibitor Sets

Ensure the integrity of purified proteins for downstream analysis and accurate characterization by using protease inhibitor cocktails and highly specific protease inhibitors. During protein expression and isolation, endogenous proteases rapidly begin to degrade protein samples, reducing the quality and quantity of protein samples required for characterization and analysis. By using the right combination of protease inhibitors, you can protect your purified protein preparations from the most common proteases including serine proteases, metalloproteases, cysteine proteases, aminopeptidases, and aspartic proteases.

Key Features:

- Convenient--Flexible protocol and ready-to-use formulations
- Consistent--High quality ensures reproducibility and excellent inhibition over a wide range of protease classes
- Flexible--Comprehensive selection of specific cocktail formulations designed to inhibit proteolytic activity from most tissue or cell type extracts, including mammalian, bacterial, yeast, fungal, and plant cells
- Application-Specific--Available without EDTA for purification schemes involving metal ion chelating
- Chromatography or analysis using 2-D gel electrophoresis. New protease inhibitor cocktail formulations include recombinant aprotinin for applications that require the use of animal-free reagents

Recommended Application	Protease Inhibitor Cocktail	Catalogue No.
General use	Protease Inhibitor Cocktail Set I	539131
Bacterial cell extracts (except those intended for metal chelation chromatography)	Protease Inhibitor Cocktail Set II	539132
Mammalian cells and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2-D gel electrophoresis	Protease Inhibitor Cocktail Set III, EDTA-Free	539134
Mammalian cells and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2-D gel electrophoresis	Protease Inhibitor Cocktail Set V, EDTA Free	539137
Proteins containing His•Tag® sequences	Protease Inhibitor Cocktail Set VII	539138

For more information, visit www.emdbiosciences.com/ProteaseInhibitors

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available at emdbiosciences.com

Description	Catalogue No.
BugBuster 10X Protein Extraction Reagent	70921
BugBuster Plus Benzonase Nuclease	70750
YeastBuster Protein Extraction Reagent	71186
CytoBuster Protein Extraction Reagent	71009
NucBuster™ Protein Extraction Reagent	71183
Protease Inhibitor Cocktail Set II (for bacterial cell extracts)	539132
Protease Inhibitor Cocktail Set VII (for proteins containing His•Tag sequences)	539138

To place your order or for complete product information visit www.emdbiosciences.com

X. Protein Purification

Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. EMD Millipore offers a broad portfolio of tools for affinity purifying tagged recombinant proteins, immunoprecipitation, and albumin/IgG depletion from serum or plasma. For extremely fast and easy protein purification, trust our PureProteome™ magnetic bead purification systems, which feature low nonspecific binding and minimal sample loss.

PureProteome beads have high binding capacity and are captured more efficiently by our unique magnetic stands.

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Description	Catalogue No.
PureProteome™ Nickel Magnetic Beads	LSKMAGH10
PureProteome Magnetic Stand, 8 well	LSKMAGS08
PureProteome Magnetic Stand, 15 mL	LSKMAGS15

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Description	Catalogue No.
S-protein Agarose	69704
GST•Mag™ Agarose Beads	71084

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XI. Protein Concentration and Buffer Exchange

Each protein preparation is unique. Give it the special treatment it deserves with a perfectly designed device for concentration and buffer exchange. Choose the right device from EMD Millipore's portfolio of sample concentration tools, featuring the Amicon Ultra line of centrifugal filters and D-Tube™ Dialyzers.

- Amicon Ultra centrifugal filters give you the fastest, most efficient concentration for most sensitive downstream applications
- Find the right filter to concentrate your sample -- search with our NEW online Amicon Ultra Selector Tool. Visit: www.millipore.com/FastEasy2
- D-Tube Dialyzers give you a gentle way to concentrate intractable or sensitive samples and prevent them from precipitation or over-concentration.
- For D-Tube ordering information visit www.emdbiosciences.com/dtube

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