product spotlights

GC5™, GC10™, and Thunderbolt™ Competent Cells

High efficiency chemically competent and electrocompetent cells.

Three new powerful competent cells have been added to Sigma's current selection of JM109, HB101, and the BL21 family. GC5 and GC10 are chemically competent *E. coli* cells, while Thunderbolt is an electrocompetent version of GC10. These cloning strains are comparable to other popular cell lines, while providing the highest efficiencies available. GC5 and GC10 carry *recA1* and *endA1* mutations that aid in plasmid stability and improved quality of prepared plasmid DNA. GC10 competent cells are also suitable for transformation of methylated DNA.

Features and Benefits

- Comparable to popular DH5α™ and DH10B™ strains
- Highest efficiencies: 1 x 10^9 to 1 x 10^{10} CFU/µg DNA
- Blue-white selection
- T1-bacteriophage resistant for protection of libraries
- Single reaction and value formats

Ordering Information

Product	Description	Unit
G 3169	GC5 Competent Cells, Uni-Pack	10 x 50 µl 20 x 50 µl
G 3044	GC5 Competent Cells, Standard Aliquots	5 x 200 µl
G 2919	GC10 Competent Cells, Uni-Pack	10 x 50 µl 20 x 50 µl
G 2794	GC10 Competent Cells, Standard Aliquots	5 x 200 µl
T 7699	Thunderbolt GC10 Electrocompetent Cells	5 x 80 µl 5 x 100 µl

See coupon flyer for special offer.

GC5, GC10, and Thunderbolt are trademarks of GeneChoice,® Inc.
DH5α and DH10B are trademarks of Invitrogen.

JumpStart™ RED HT RT-PCR Kit

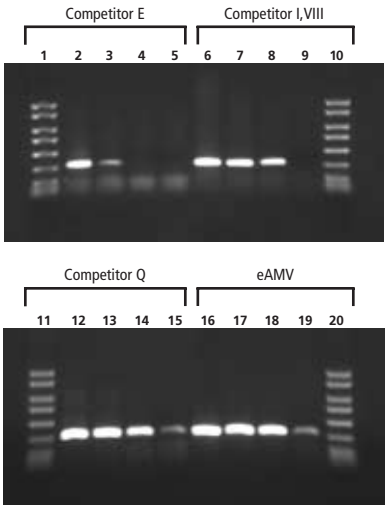
A novel high-throughput solution for gel-based RNA detection.

The JumpStart RED HT RT-PCR Kit is designed for high throughput gel-based RT-PCR applications, optimized for end-point detection of target RNA in a complex sample matrix. The kit contains a combination of Sigma's enhanced avian myeloblastosis virus reverse transcriptase (eAMV-RT™) and JumpStart RedTaq™ DNA polymerase.

Features and Benefits

- Greater transcription efficiency
- Enhanced sensitivity and specificity
- Direct sample loading after PCR
- Maximum flexibility and minimum optimization

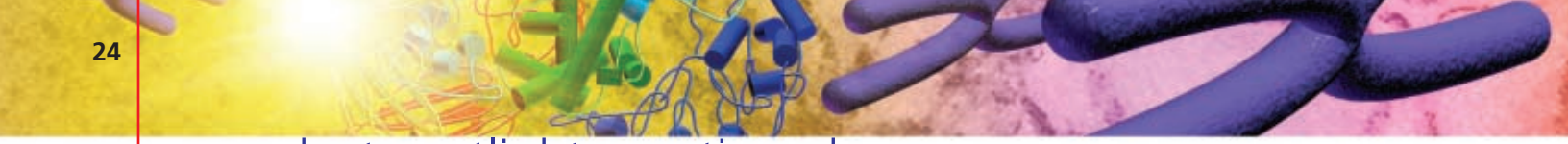
eAMV has superior performance and thermal stability
Sensitivity for low abundance message is critical for comparative gene expression studies. eAMV has superior processivity and thermal stability compared to other reverse transcriptases. These unique features make it the ideal enzyme for detection of low abundance RNA, especially those with difficult secondary structure.



RT-PCR was performed on total RNA-HELA cells serially diluted from 1000 ng to 1 ng.
Reactions were performed with β-actin primers according to suppliers' protocols.
Lanes 1, 10, 11, 20: *PCR Marker*
Lanes 2, 6, 12, 16: *1000 ng*
Lanes 3, 7, 13, 17: *100 ng*
Lanes 4, 8, 14, 18: *10 ng*
Lanes 5, 9, 15, 19: *1 ng*

Ordering Information

Product	Description	Unit
J 3520	JumpStart RED HT RT-PCR Kit	40 reactions 200 reactions



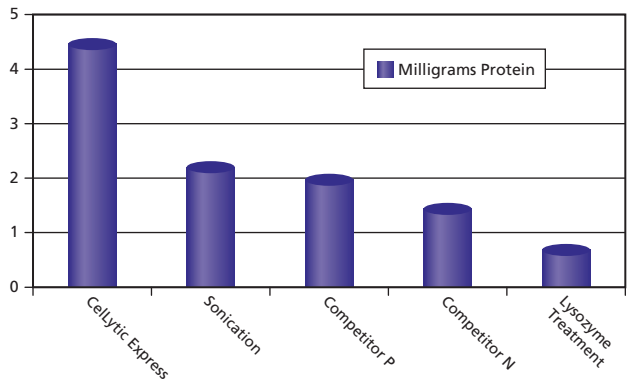
CelLytic™ Express

CelLytic Express is a proprietary blend of detergents and enzymes used to extract proteins from bacterial cells by lysis, directly in culture medium. There is no need for special equipment such as a sonicator or a French press. The one-step extraction method eliminates the need for cell harvest or clarification of lysates prior to purification, allowing for direct affinity adsorption of the target proteins to resin from a total culture extract.

With CelLytic Express intact fusion proteins have been successfully purified using HIS-Select™ and Anti-FLAG M2™ affinity purification resins. When CelLytic Express is used with HIS-Select or glutathione magnetic beads, the entire culture, extraction, and purification process can be accomplished directly in the culture flask or tube. CelLytic Express is provided as a ready-to-use, all-in-one formulation, which does not require the addition of separate reagents for optimal protein extraction. In-culture cell lysis routinely results in higher yields than traditional extraction methods, which also require time-consuming cell harvest steps.

Features and Benefits

- Save time by eliminating cell harvest and clarification steps – no centrifuge needed
- Higher recombinant protein yields when using CelLytic Express whole culture lysis
- All-in-one formula is optimized for protein extraction without additional reagents



Target Protein Recovery. *Ten milliters of a Terrific Broth (Product Code T 9179) culture containing BL21 E. coli cells expressing a 27 kDa histidine-tagged protein were lysed and subsequently purified using HIS-Select Nickel Affinity Gel (Product Code P 6611). CelLytic Express lysis was performed directly in the culture. For other methods, cells were first harvested by centrifugation before mechanical, detergent, or enzymatic lysis. Total protein recovery was determined by Bradford assay of the elution pools from each lysis method.*

Ordering Information

Product	Description	Unit
C 1990	CelLytic Express	6 x 500 ml 10 x 25 ml

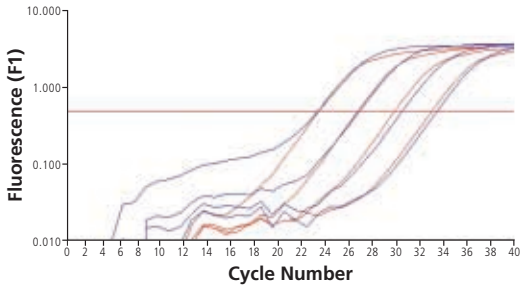
Quantitative Reverse Transcriptase PCR ReadyMix™ for Probe-Based Applications

Quantitative Reverse Transcriptase PCR (QRT-PCR) provides a highly sensitive method for the quantitative analysis of gene expression. Sigma's QRT-PCR ReadyMix combines the advantages of Enhanced Avian Reverse Transcriptase (eAMV™ RT) and JumpStart Taq with a ready-to-use mix specifically designed for probe-based QRT-PCR. Regardless of difficult secondary structures or chosen fluorescent detection chemistry, QRT-PCR ReadyMix is specially formulated to help you achieve superior results.

Features and Benefits

- ***Greater Specificity & Increased Target Yield*** – Inactivated by a bound antibody, Sigma's JumpStart Taq enzyme decreases non-specific amplification while enhancing target yield
- ***Maximum Versatility*** – Compatible with a variety of fluorescent detection methods, including dual-labeled probes, Molecular Beacons™, or double-stranded binding dyes such as SYBR® Green I, and has the ability to transcribe through difficult secondary structures at elevated temperature (up to 65 °C)
- ***Convenience with a ReadyMix*** – Reduce set-up time, eliminate contamination concerns, and non-specific amplification, simply add template, primers, and fluorescent detection chemistry

QRT-PCR ReadyMix for probe-based applications is a 2x concentrate blend of JumpStart Taq, 99% pure dNTPs, buffer, glass passivator, and stabilizers. The ReadyMix is also packaged with a separate vial of eAMV RT, 25 mM $MgCl_2$, 10x PCR buffer, and Reference Dye for normalization of the reaction data.



Ordering Information

Product	Description	Unit
QR0200	Quantitative RT-PCR ReadyMix for probe-based applications	1 kit

Autoclavable Clonal Selection Media with S-Gal™

A black-white color selection reagent superior to X-Gal.

S-Gal is a unique chromogenic substrate for β-galactosidase in traditional gene insertion analysis. In only 14 hours, black S-Gal-stained colonies are already much easier to distinguish from unstained colonies than in X-Gal-mediated blue-white color selection. Unlike X-Gal, S-Gal is a non-toxic water-soluble reagent that is not light-sensitive and may be autoclaved or microwaved. These useful features provide superior ease in preparation of selection media, and, as a result, we are able to provide premixed media for additional convenience and time savings.

Features and Benefits

- Superior to X-Gal for clonal selection
- Faster, sharper colony development
- Can be autoclaved or microwaved
- Convenient premixed media available
- Safe, non-toxic water-soluble reagent

S-Gal/IPTG LB agar vs. standard X-Gal/IPTG agar. Left: *S-Gal plates were prepared by simply mixing the S-Gal/IPTG LB agar blend with water, autoclaving, and pouring the plates.* ***Right:*** *X-Gal plates were prepared by first dissolving X-Gal in dimethylformamide and dissolving/filtering an IPTG solution. The LB agar was mixed with water, autoclaved, and cooled to 50 °C. Finally X-Gal and IPTG were added to the medium and the plates were poured. The plates were spread with E. coli transformed with pUC18 and pFLAG-CMV™-1-BAP (no β-galactosidase gene). The photograph was taken at 23 hours post-plating.*

Ordering Information

Product	Description	Unit
C 4478	S-Gal/LB Agar Blend with IPTG	500 ml 6 x 500 ml
S 9938	S-Gal/LB Agar Blend (without IPTG)	500 ml 6 x 500 ml
S 1813	S-Gal/Kanamycin/LB Agar Blend with IPTG	500 ml 6 x 500 ml
S 7313	S-Gal Sodium Salt (Water Soluble)	100 mg 1 g 5 g
S 9811	S-Gal (Soluble in DMF or DMSO)	100 mg 1 g 5 g

See coupon flyer for special offer.

TnT®, T7 Coupled Wheat Germ Extract System

Standard wheat germ extract translations commonly use RNA synthesized *in vitro* from SP6, T3 or T7 RNA polymerase promoters.[1,2] This entire process requires separate reactions with several steps between each reaction. The TnT Extract System bypasses many of these steps by incorporating transcription directly in the translation mix. Additionally, the TnT Extract reactions often produce significantly more protein (2- to 6-fold) in a 1.5-hour reaction than do standard *in vitro* wheat germ extract translations using RNA templates. With this system, an *in vitro* transcription and translation reaction can be completed in 1.5 hours with incubation at 30 °C using 0.2-2 µg of DNA template.

The following templates can be used with this system:

- Assembled templates from *in vitro* Director™ PCR System (5′ FLAG, 5′ c-Myc, 3′ FLAG, or 5′ Anchor)
- Linearized plasmid DNA containing a T7 RNA polymerase promoter
- Circular plasmid DNA containing both a T7 RNA polymerase promoter and T7 transcription terminator

Potential applications of the TnT T7 Coupled Wheat Germ Extract System include:

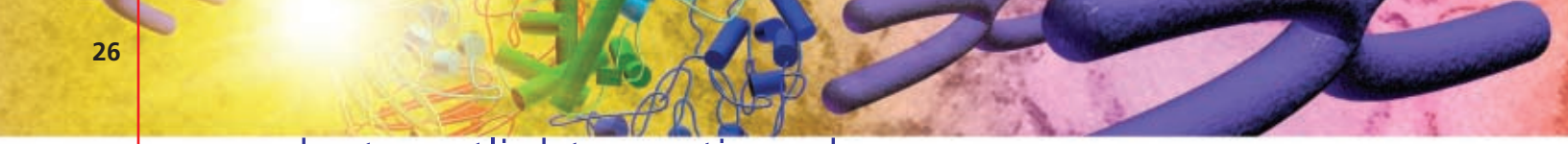
- Truncation mutation analysis
- Mutation detection and analysis
- Protein-protein interactions
- Immunoprecipitation of protein complexes
- Protein dimerization assays
- Ligand-binding region determination
- Protein structure analysis
- Electrophoretic mobility shift assays for DNA-protein interactions
- DNA footprinting and protein-crosslinking studies
- Protein-RNA binding assays
- *In vitro* expression cloning

References

1. Anderson, C.W. et al. Preparation of a Cell-Free Protein-Synthesizing System from Wheat Germ. *Methods Enzymol.* **101,** 635 (1983).
2. Krieg, P.A. and Melton, D.A., Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs. *Nucleic Acids Res.* **12,** 7057 (1984).

Ordering Information

Product	Description	Unit
TN0100	TnT T7 Coupled Wheat Germ Extract	1 kit



New Desthiobiotin Polyethyleneoxide Iodoacetamide for Purification of Cysteine-containing Peptides in Proteomics Applications

Sigma-Aldrich has introduced new Desthiobiotin Polyethyleneoxide (PEO) Iodoacetamide (Product Code D 2192), a water soluble, thiol-specific biotinylation reagent, designed for the modification of reduced cysteine residues in proteins. Following labeling with this reagent, a proteome digest is easily fractionated by capture with an appropriate streptavidin or avidin-based affinity matrix. The desthiobiotinylated peptides are then quickly and quantitatively recovered using mild conditions of competitive elution. The result is a significantly simplified mixture that provides for easier identification of individual proteins or peptides. Desthiobiotin PEO Iodoacetamide has demonstrated utility in thiol labeling in a variety of proteomics applications, such as peptide mapping and mass spectrometry.

Features and Benefits

- Cysteine-specific labeling reagent for affinity purification
- Easy and efficient elution from streptavidin matrices with millimolar concentrations of biotin or desthiobiotin at neutral pH
- Water soluble

Ordering Information

Product	Description	Unit
D 2192	Desthiobiotin Polyethyleneoxide Iodoacetamide	50 mg

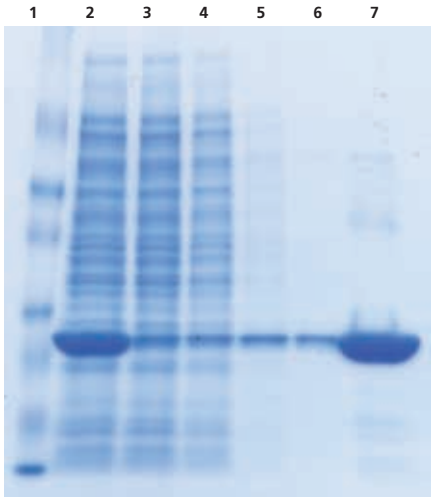
Related Products		
S 6940	SigmaScreen™ Streptavidin High Capacity (HC) Coated plate, 96 well, clear	1 each 5 x 1 each
S 1638	Streptavidin Agarose	1 ml 5 ml
E 5529	EZview™ Red Streptavidin Affinity Gel	1 ml 5 x 1 ml

HIS-Select™ Magnetic Agarose Beads

HIS-Select Magnetic Agarose Beads (Product Code H 1786) consists of paramagnetic, immobilized metal-ion affinity chromatography (IMAC) resin, designed for use in automated and small-scale affinity capture purifications. The HIS-Select Magnetic Agarose Beads bind His-tagged recombinant proteins to allow their purification from cell lysates and other biochemical solutions. The His-tagged proteins, bound to the affinity resin, are separated with the use of a magnet. The magnetic properties allow for very rapid processing and aid in manipulations such as repetitive washings and recovery of protein-bound beads. This leads to faster recovery, experimental reproducibility, and more accurate quantitation of the proteins of interest.

Features and Benefits

- Improved specificity over competitor products
- Flexible – small-scale to high throughput
- Binding capacity greater than 15 mg/ml



Lysates from E. coli containing a histidine-tagged protein were purified using 50 µl HIS-Select Magnetic Agarose Beads. *Wash buffer contained 50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidizole, pH 8.0. Eluted with 50 mM sodium phosphate, 300 mM sodium chloride, 250 mM imidazole, pH 8.0.*

Lane 1: *Sigma ColorBurst™ (Product Code C 4105)*
Lane 2: *Cell Extract*
Lane 3: *Flow Through*
Lane 4: *Wash 1*
Lane 5: *Wash 2*
Lane 6: *Wash 3*
Lane 7: *Eluate*

Ordering Information

Product	Description	Unit
H 1786	HIS-Select Magnetic Agarose Beads	2 x 1 ml 5 x 1 ml

Glutathione High Capacity Coated Plates Clear, 96-Well

Sigma-Aldrich recently introduced a Glutathione High Capacity Coated Plate for high-throughput, single-step purification of glutathione-binding proteins. The clear 96-well coated plates are coated with a proprietary, patent pending high-density matrix containing glutathione. The plates are supplied in a ready-to-use format with no blocking steps necessary.

The plates bind greater than 2 µg of glutathione-binding protein, providing ample material for downstream analysis. The plates offer low well-to-well and plate-to-plate variability with CVs of less than 15%.

Features and Benefits

- Single-step purification of glutathione-binding proteins
- High-throughput format
- Sufficient capacity for various analytical methods to include: SDS-PAGE, mass spectrometry, and protein quantitation

0.2 ml of a clarified lysate from E. coli expressing glutathione-binding protein was added to the wells of a Glutathione HC plate and allowed to incubate overnight at 25 °C. *The wells were washed and eluted with 0.2 ml of Laemmli sample buffer and loaded onto a 4-20% Tris-glycine gel. The gel was silver stained using the ProteoSilver Silver Stain Kit (Product Code PROT-SIL1). Note that the silver stain shows no non-specific proteins after elution (Lane 2).*
Lane 1: *E. coli lysate containing glutathione binding protein (Load)*
Lane 2: *5% of well after elution with Laemmli sample buffer*

Ordering Information

Product	Description	Unit
G 3294	Glutathione High Capacity Coated Plates, Clear, 96-Well	1 each 5 x 1 each

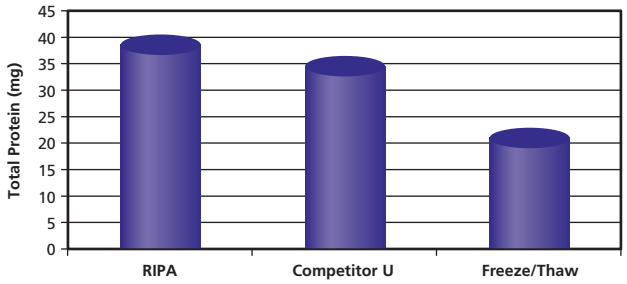
RIPA Buffer

Extraction of cellular proteins requires efficient cell lysis and protein solubilization, while avoiding protein degradation and/or interference with protein immunoreactivity and biological activity.

RIPA (**R**adio-**I**mmuno**p**recipitation **A**ssay) Buffer (Product Code R 0278) is designed for rapid, efficient cell lysis and solubilization of proteins from both adherent and suspension-cultured mammalian cells. It has long been a widely used lysis and wash buffer for small-scale affinity pull-down applications, such as immunoprecipitation, since most antibodies and protein antigens are not adversely affected by this buffer. In addition, RIPA Buffer minimizes non-specific protein binding, which keeps background low, while allowing most specific interactions to occur, enabling studies of relevant protein-protein interactions. One ml of the RIPA Buffer is sufficient to lyse cells from one 100-mm culture dish (0.5 to 5 x 10^7 cells) of most adherent mammalian cell lines.

Features and Benefits

- Formulation: 150 mM NaCl, 1.0% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0
- Ready-to-use, efficient lysis buffer
- Compatible with protease and phosphatase inhibitors
- Low background
- Tested to be free from protease contamination
- Allows specific protein binding interactions with minimum of non-specific binding
- Compatible with EZview™ Red Affinity Gel product line
- Comprehensive technical bulletin



Comparison of protein extracted using Sigma RIPA Buffer, Competitor U RIPA buffer, and the freeze/thaw method of cell lysis. *CHO K-1 cell suspension cultures (approximately 1 x 10^6 cells each) were spun down to remove the media. 300 ml of the appropriate buffer was added to each cell pellet. For the freeze/thaw method, the buffer used was 50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0. The other lysates were made using the lysis buffers indicated. Each sample was allowed to mix for 15 minutes before being centrifuged to remove cellular debris. The cleared lysate supernatants were analyzed for protein concentration. Protein concentrations were determined by BCA protein assay (Product Code BCA-1). As shown in the graph, the RIPA Buffer extracted more protein than the competitor and the freeze/thaw methods.*

Ordering Information

Product	Description	Unit
R 0278	RIPA Buffer	50 ml 500 ml