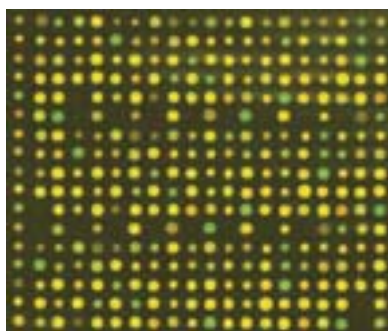


Biochemistry & Molecular Biology Analysis

Protein expression study



Efforts to understand the relationship between protein structure and biological function have intensified, and the huge number of candidate proteins generated by genomic programs has generated interest in all aspects of protein expression. Study methods include typically micro arraying and cell expression systems.

- ◆ **Protein microArray** is established as a powerful means to detect proteins, monitor their expression levels, and investigate protein interactions and functions. It allows screening a large number of samples with definite probes, or a limited number of samples against a large number of probes.
- ◆ **Cell expression systems** (genetically transformed with vectors, or transfected with) allow introducing, inducing or depleting protein expression in living cells to study their localization, expression level and dynamics, in-vivo modifications. They also allow producing recombinant proteins, generally purified from cells and culture supernatants by immunoaffinity or other ligand affinity methods in high quantity and homogeneity.

Interchim has :

- ◆ Powerful microarray products (Nexterion slide H to prepare your own arrays, and Labvision Antibody arrays to screen for hundreds of specificities)
- ◆ Human Tissue Array & Single Slides
- ◆ Reporter assays and in vivo protein expression (GFP, α -Glucuronidase)
- ◆ Recombinant proteins systems (UptiTherm™ Cloning and Expression Kit, plasmids)
- ◆ Silencing transfection reagents (TKO, siRNA)

Protein Microarray

Nexterion slide H

Key Product Features

- ◆ Ideal substrate for spotting peptide, protein, or cell/tissue microarrays
- ◆ High loading capacity
- ◆ Very low non-specific binding characteristics
- ◆ Optimal preservation of native structure and biological activity of protein probes
- ◆ High signal intensities even from low abundant proteins or weakly expressed genes
- ◆ Compatible with common microarrays (contact and non-contact) and scanners

Nexterion® Slide H is ideally suited for covalent immobilization of peptides and proteins (including functional proteins such as antibodies, antibody fragments, enzymes, or receptors), and even cell or tissues. In addition, amino-modified oligonucleotides (size d 25 mers) can also be immobilized on the slide surface. The permeable, 3D hydrogel coating preserves the native three-dimensional structure of proteins thereby maintaining protein stability and functionality. Nexterion® Slide H enables excellent signal-to-background ratios and exceptionally high linear dynamic ranges compared to conventional 2D coatings by a unique combination of low non-specific binding characteristics, high loading capacity, and the use of glass with low auto-fluorescence. Even very low intensity signals, such as those from low-abundance proteins or weakly expressed genes can be reliably detected and quantified. The density of the reactive groups is consistent over the entire surface and is optimized to maximize binding capacity. The reactive chemistry is stable and remains active even during very long spotting runs.

Description	Cat.#	Qty
Nexterion slide H, 75.6 mm x 25 mm	U9194D	25 units
Related products : labeling agents		
FluoProbes 547 NHS ester (557/574 nm)	FP-AK7730	1 mg
FluoProbes 647 NHS ester (652/673 nm)	FP-AK7740	1 mg

Cellular microarray based on Nexterion® Slide H (Confocal Microscopy)
Cell attachment was mediated by a peptide and polyLysine. In courtesy of Dr. Roland Brock, Eberhard-Karls-University, Tuebingen, Germany.

See also application for oligonucleotides coating in section Genomics/MicroArrays page D117.

Antibody arrays

Antibody Arrays allow the researcher the convenience of detecting and analyzing hundreds of proteins simultaneously on a single slide; saving time, reagents, and more importantly, reducing the number of variables that influence experimental outcome. Proteins from cell extracts, tissue lysates or treated samples may be used for analysis and detection.

Using Antibody Arrays, one is able to determine qualitative and not quantitative measures of protein expression. Antibody Arrays aid in determining relative expression levels, comparing gene expression profiles of normal, diseased or treated samples; or understanding expression patterns of various proteins in a given sample.

Antibody Array platform consists of 700 antibodies printed in triplicate on nitrocellulose-coated slides or on glass slides, in a set of two slides. Lab Vision antibodies are characterized for western blot, immunoprecipitation and immunohistochemistry, adding value to your research. The antibodies cover research interest from signal transduction, apoptosis, angiogenesis, cell cycle, transcriptional regulation, to cancer markers and much more.

Features

- ◆ More than 700 antibodies in triplicate per slide
- ◆ Two slides per set
- ◆ Very competitive prices
- ◆ 1 year shelf life
- ◆ Efficient, and consistent results

The following antibody groups are incorporated into the antibody array.

Adhesion Molecule	Angiogenesis	Apoptosis
Cancer Markers	Cell Cycle	Cell Junctions
Cytoskeletal Proteins	DNA replication & repair	EGFR family
Epitope Tags	Extracellular Matrix Proteins	G Proteins & G-protein coupled receptors
Hematopoiesis	Hormones	Immunoglobulins
Inflammation	Insulin homeostasis	Ion Channels
Negative	Controls Neuroscience	Nuclear Receptors
Proteases & Inhibitors	Proteolysis Receptor	Tyrosine Kinase
Stem Cell	Stress Response	T-cell Markers
TNFR family	Transcriptional Regulators	Transferrin Receptor
Tumor suppressors		

Description	Cat.#	Qty
Antibody arrays 2 slides, supplied with the white detection system and chamber (Cy3™ based)	BS9780	1 kit

Human Tissue Array & Single Slides

Human single-tissue section slides are 5 µm thick, formalin-fixed, paraffin-embedded tissue sections mounted on microscope slides and are used for *in situ* gene expression analysis by *in situ* hybridization and immunohistochemistry.

The human single-tissue section slides may be deparaffinized with a xylene-alcohol series and rehydrated in water and treated with target retrieval procedures if required. Conventional immunohistochemical and *in situ* hybridization reactions are then carried out.

The human tissue array tissue sections are 5-10 µm in thickness and mounted on positively-charged glass slides. Each tissue sample is about 2mm in diameter. A reference chart shows all the tissue names and their respective position on the array. This product can be used for both immunohistochemistry and *in situ* hybridization. It is an ideal product for rapid cellular localization of RNA and protein expression.

Each antibody on the slide may be purchased separately for further evaluation. Please inquire for Ab specificity content of each microArray, or for customized arrays with your own antibodies.

Related product
TMA Builder **BU8050**



Device for producing TissueMicroArray.

Also available : Animal Model Tissue Microarrays

Mouse whole body screen 1	BU8070
Rat	BU8080
Dog	BU8090
Primate	BU8100

*Each array includes 2 specimens from 2 different individual for up to 20 tissue types.

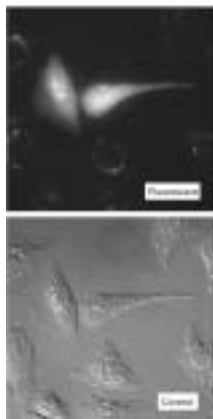
*Large elements (3,5 mm) formalin-fixed, paraffin-embedded.

See also ReadyBlot Protein explorer® (Kidney, brain)

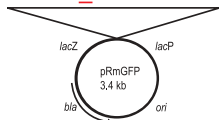
Biochemistry & Molecular Biology Analysis

Protein expression study

B.212



GGG TTC AAA GTG AGT AAA CAA ATA TTG AAG AAC ACT TGT TTA GAA GAA GTA ATG TCG TAT
AAA GTA AAT CTG GAA GGA ATT GTA AAC AAC CAT GTT TTT ACA ATG GAG GGT TCG GGC AAA
GGG AAT ATT TTA TTC GGC AAT CAA CTG GTT CAG ATT CGT GTC ACG AAA GGG GCG CCA CTG
CCT TTT GCA TTT GAT ATT GTG TCA CCA GCT TTT CAA TAT GGC AAC GGT ACT TTC ADG AAA
TGT CCG AAT GAT ABA TCA GAT TAT TTT ATA CAA TCA TTT CCA GCA GGA TTT ATG TGT GAA
CGA ACA TTA CGT TAC GAA GAT CCG GGA CTT GTT GAA ATT CGT TCA GAT ATA AAT TTA ATA
GAA GAC AAG TTC GTC GTC TAC ACG GTG GAA TAC AAA GAT AGT AAC TTC CCA GAT GAT GGT ACC
GTC ATC CAG AAC ACT ATC TTA GGA ATG GAG GCT TAC TTT GAA GGC ATG TAC ATG AAT AAT
GGC GTG TTG GTC GGC GAA GTA ATT CTT GTC TAT AA CTA AAC TCT GGG AAA TAT TAT TCA
TGT CAC ATG AAA ACA TTA ATG AAG TCG AAA GGT GTA GTA AAG GAG TTT CCT TCG TAT CAT
TTT ATT CAA CAT CGT TTT GAAA GACT TAC GTA GAA GAC GGG GGT TCT GTT GAA GAG CAT
GAG ACT GCT ATT GCT CAA ATG ACA TCT ATA GSA AAA CCA CTA GGA TCC TTA CAC GAA TGG
GTT CAT CAC GAT CAC CAT CAC TCA TCT AGH



Product	Cat. # (5/pkg)
Human Tissue	
Adrenal Gland	FM2660
Alzheimer's Brain	AY5310
Amvloidosis of Kidney	FM2940
Analcan	FM3070
Angiosarcoma	FM2920
Aorta	FM3060
Appendix	FM2490
Astrocytoma	FM2430
Bladder Carcinoma	FM2790
Bone Marrow	FM2890
Brain	FM7530
Brain Tumor	FM2770
Breast Carcinoma (ER-/PgR-)	FM2730
Breast Carcinoma (ER+/PgR+)	FM2740
Breast Normal	FM2850
Cerebellum	FM2540
Cervical Lymph Node (ALK/p80+)	FM2870
Colorectal Carcinoma	FM2640
Endometrial Ca.	FM2990
Endometrium Normal	FM2980
Epstein-Bar Virus infected	FM2620
Esophagous	FM2930
Fetal Liver	FM2680
Glioblastoma	FM2700
Glioma	FM2830
Heart Tissue Sections	FM2560
Heart, left ventricule	FM3010
Heart, right ventricule	FM3000
Hepatitis B Virus Infected Liver	FM2460
Hilar Lymph Node (ALK/p80+)	FM2860
Hipocampus	FM3020
Hodgkin's Lymphoma	FM2450
Hydatid Mole	FM2960
Hypothalamus	FM3030
Ileum	FM3080
Kidney	FM2550
Leiomyoma	FM2650
Liver	FM2720
Liver Ca.	FM2810
Lung	FM2610
Lung Ca.	FM2820
Lymph node	FM2590
Lymphoma	FM2760
Mantle's Lymphoma	FM2970
Melanoma	FM2600
Mesothelioma	FM2480
Ovarian Carcinoma	FM2530
Pancreas	AY5300
Parathyroid-hyperplasia	FM3040
Pelvic	FM2900
Phabdomyosarcoma	FM2440
Pituitary	FM2690
Placenta	AM7540
Prostate	FM2410
Prostate Carcinoma	BM2360
Schwannoma	FM2500
Seminoma	FM3050
Skeletal Muscle	FM2420
Skin	FM2510
Small Intestine	FM2520
Spine	FM2880
Spleen	FM2580
Stomach	FM2470
Stomach Carcinoma	FM2750
Stromal Tumor	FM2910
Submandibo	FM3090
Testis	FM2950
Thymus	FM2670
Thyroid	FM2570
Thyroid Ca.	FM2800
Tonsil	FM2630
Uterus Ca.	FM2840
Uterus Leiomyoma	FM2780
Vein	FM3100
Wilim's Tumor	FM2710
Human Tissue Array	Cat.# (2/pkg)
Array Breast tumor (64 spots)	BM2370
Colon Tumor Tissue Array (64 spots)	BM2380
Lung Tumor Tissue Array (64 spots)	BM2390
Normal & Tumor Tissue Array	BM2400 (30 normal human spots, 41 common tumor spots)
Ovary Tumor Tissue Array (64 spots)	BM2410
Rectum Tumor Tissue Array (64 spots)	BM2420



Reporter assays and in vivo protein expression

Fluorescent proteins have a diverse range of uses in from cell biology, drug discovery, biomedical research; high throughput screening (HTS) and fluorescence activated cell sorting (FACS). Fluorescent proteins expressed in living cells (e.g. fungi, bacteria, animals and plants) can be used to study molecular processes in real time such as dynamics of nuclear division, protein trafficking, etc. GFPs can also be used for the dynamic monitoring of protein interactions using FRET or BRET technologies.

Green Fluorescent Proteins

Functional analysis of protein

- ◆ Experiments indicate that *in vitro* the *Renilla* GFP's are over 3 times brighter than *Aequorea* eGFP, the most popular GFP used by scientists.
- ◆ Experiments *in vivo* also indicate that these *Renilla* GFPs are also brighter and may be less cytotoxic than *Aequorea* GFP.
- ◆ Excitation and emission peaks are almost symmetrical with a relatively small Stokes shift
- ◆ Successful expression has been achieved in bacteria, fungi and mammalian cells (Hela, Cos1 and CHO).

Renilla GFP Vectors are available as native gene for expression in bacterial cells (pUC) and codon optimized for expression in mammalian cells (pGEX-4T), but they also express well in fungi.

Description	Cat.#	Qty
<i>Renilla mullerei</i> native GFP in pUC19 backbone	FP-AM6840	25 µg
<i>Renilla reniformis</i> native GFP in pUC18 backbone	FP-AM6850	25 µg
<i>Renilla mullerei</i> humanized GFP in pGEX-4T backbone	FP-BL8680	25 µg

pLIVE™ In Vivo Expression Vectors

Designed for high level, prolonged expression of transgenes in the mouse

The pLIVE™ (Liver *In Vivo* Expression) Vector is designed for high level, prolonged expression of transgenes in the mouse liver after hydrodynamic tail vein delivery. This vector utilizes a chimeric promoter composed of the minimal mouse albumin promoter and the mouse alpha fetoprotein enhancer II. Two introns have been engineered into the expression plasmid so that they will be present in the primary transcript produced from the liver-specific chimeric promoter, in turn increasing expression of the delivered transgene.

Downstream of the first intron is a multiple cloning site (MCS) with eight unique restriction sites allowing for simple insertion of the gene of interest. Together the chimeric promoter and the two introns are capable of promoting high level cloned transgene expression in the liver for extended lengths of time compared to classic promoters such as the cytomegalovirus immediate early promoter (CMV).

In addition to the pLIVE™ Vector, two reporter vectors derived from pLIVE™, pLIVE™-*lacZ* and pLIVE™-SEAP, are available for use as positive controls. Expression of the *E. coli lacZ* gene from pLIVE™-*lacZ* can be monitored in the liver using either classical X-gal staining of liver tissue sections, or quantitative β-galactosidase assays using liver lysates. Expression of human placental secreted alkaline phosphatase (SEAP) from pLIVE™-SEAP can be easily monitored using a quantitative assay of mouse serum. The high level, long term liver-specific expression of transgenes from the pLIVE™ Vector, as well as the availability of the positive control pLIVE™-*lacZ* and pLIVE™-SEAP reporter vectors, make the pLIVE™ Vectors the ideal choice for *in vivo* liver expression studies in mice.

Description	Cat.#	Qty
pLIVE™ Vector	BM4450	20 µg
pLIVE™ Vector/LacZ Control Vector Kit	BM4460	20 µg of each
pLIVE™ Vector/SEAP Control Vector Kit	BM4470	20 µg of each
pLIVE™ Vector Complete System (All 3 Vectors)	BM4480	20 µg of each

Related products

TransIT®-QR Hydrodynamic Delivery Solution	BM4530
Beta-Gal Staining Kit	J29660

Technical tip

Fluorescent proteins

Renilla reniformis (Sea Pansy) and *Renilla mullerei* (Gulf of Mexico) are species of soft coral. The organism is a colony of polyps each of which is bioluminescent at the sites identified by the characteristic green fluorescence.

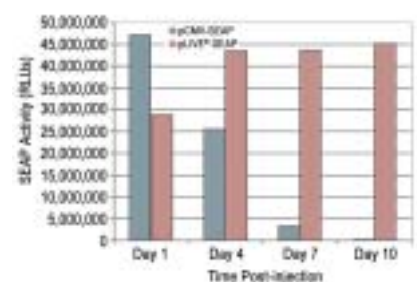
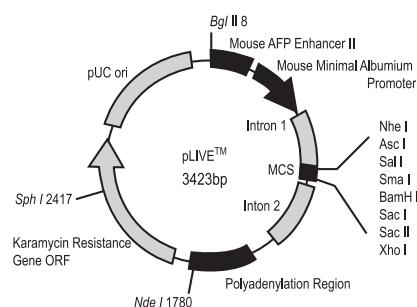
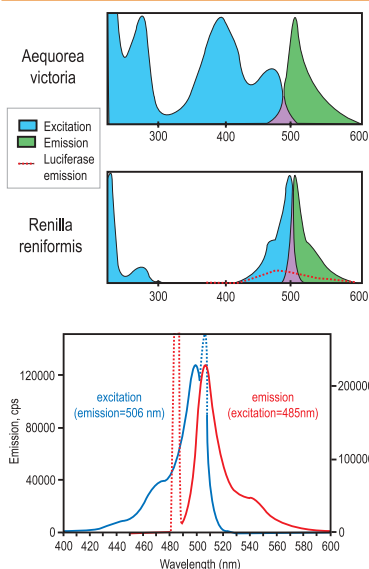


Figure 2.

The Promoter in the pLIVE Vector Outperforms the CMV Promoter for Long-term Liver Expression. The pLIVE-SEAP Vector (20 µg) and pCMV-SEAP (10 µg, CMV promoter driven SEAP vector) were each delivered to four ICR mice using the hydrodynamic tail vein injection procedure and the TransIT®-QR Hydrodynamic Delivery Solution (BM4530). At the indicated days post-injection, serum from each mouse was collected and the level of SEAP activity present was determined using the Phospha-Light SEAP Assay Kit (Applied Biosystems). The average SEAP activity from each group is presented.

Technical tip

The β -Glucuronidase (GUS) enzyme from *E. coli* has been well documented to provide desirable characteristics as a gene marker in transformed plants. The GUS reporter gene system has many advantages including stable expression of *E. coli* GUS enzyme, no interference with normal plant metabolism, and low intrinsic GUS activity in higher plants. The enzyme is also capable of tolerating amino-terminal additions, making it useful for study plant organelle transport study.¹⁻⁴ Various β -glucuronic acid substrates are available for detection of GUS expression, all of which contain the sugar D-glucopyranosiduronic acid attached by glycosidic linkage to a hydroxyl group of a chromogenic, fluorogenic, or other detectable molecule.⁴

References :

1. Proc Natl Acad Sci USA 86, 8447 (1986)
2. Eur Mol Biol Org J 6, 3901 (1987)
3. Plant Sci 70, 130 (1990)
4. Gallagher, S.R., et al., (1992)
5. Plant Mol Biol Rep 5, 387 (1987)

Enzyme Substrates and Kits for β -Glucuronidase

See also pages D71-D75.

Phenethyl 1-thio-b-D-Glucopyranosiduronic Acid (PET-GlcU)

Inhibitor of β -Glucuronidase

This hydrophobic thio-glycoside can be used as a competitive inhibitor of β -Glucuronidase activity

Description	Cat.#	Qty
Phenethyl 1-thio-b-D-Glucopyranosiduronic Acid	FP-BM8770	50 mg

X-GLU, Substrates for glucosidase

Biotechnology grade

Substrate of β -glucosidase

5-Bromo-4-Chloro-3-Indolyl-b-D-Glucopyranoside

$C_{14}H_{15}BrClNO_6$

MW : 408.6

A substrate for β -glucosidase that renders an intense indigo-blue chromophore (615 nm) upon enzymatic action. Used as an indicator-probe in histochemistry and in culture media to detect β -Glucosidase positive organisms.

Description	Cat.#	Qty
X-GLU	UP193325	100 mg

Recombinant proteins

UptiTherm™ Cloning and Expression Kit

Dual-shuttle system that allows cloning and expression both in *E. coli* and *Thermus thermophilus*

- ◆ Applications of the kit include: cloning and expression of thermophilic proteins in a host system, thermostabilization of mesophilic proteins and direct selection of thermostable mutants in a thermophilic organism, over expression of mesophilic proteins that cannot be expressed by the usual means.
- ◆ High efficiency of pMK18 vector in transformation of *Thermus thermophilus* and other *Thermus* strains and species (e.g. *Thermus aquaticus*).
- ◆ Use of *Thermus* competent cells optimized for transformation with exogenous plasmid DNA.
- ◆ Fast screening of transformants due to kanamycine-resistance encoded by pMK18 vector.
- ◆ No recombination processes in *Thermus*.
- ◆ Exclusive incubation chamber optimized for growth of bacteria at high temperatures.
- ◆ Optimized protocol and media for efficient transformation.

Description	Cat.#	Qty
UptiTherm™ Cloning and Expression Kit	UPS54851	16 runs

Plasmid pKT1

Plasmid pKT1 is a pUC-derived plasmid containing the *kat* cassette. This cassette is expressed under the control of a promoter active in *Escherichia coli* and *Thermus thermophilus*. The cassette codifies the kanamycine resistance (30 μ g / ml). The cassette can be easily extracted by using the restriction sites included in its flanking polylinker.

Description	Cat.#	Qty
Plasmid pKT1 at 100 ng / μ l	S54830	1 μ g

Literature

Lasa et al. (1992). Mol. Microbiol., 11: 1555-1564
Patent number P9301522.

Plasmid pMK18

Plasmid pMK18 has been obtained from *Escherichia coli* and *Thermus thermophilus* sequences, containing *ori* sites. Therefore, it is a shuttle vector, that can be used for thermostabilization studies involving transformation of both organisms. The plasmid contains the *kat* cassette (see plasmid pKT1) as a selectable marker.

Description	Cat.#	Qty
Plasmid pMK18 at 100 ng / µl	S54840	1 µg

Literature :

De Grado, M. Ph.D. Thesis, UAM, Madrid, Spain
Patent number P9301522.

Plasmid pMKE1

Plasmid pMKE1 is a pUC-derived plasmid containing the *kat* cassette. The cassette codifies the *kanamycine* resistance (30 µg / ml). The cassette can be easily extracted by using the restriction sites included in its flanking polylinker. Also, the plasmid includes the *Pnar* promoter, for nitrate-induced expression. Please note that this plasmid has to be used with a special *Thermus* strain (HB27 :: nar).

Description	Cat.#	Qty
Plasmid pMKE1 at 150 ng / µl	FQ1310	1 µg

Plasmid pMKE2

Plasmid pMKE2 is a pUC-derived plasmid containing the *kat* cassette. The cassette codifies the *kanamycine* resistance (30 µg / ml). The cassette can be easily extracted by using the restriction sites included in its flanking polylinker. Also, the plasmid includes the *Pnar* promoter, for nitrate-induced expression. Please note that this plasmid has to be used with a special *Thermus* strain (HB27 :: nar).

Description	Cat.#	Qty
Plasmid pMKE2 at 150 ng / µl	BP7010	1 µg

pDream2.1/LIC Kit

Protein expression vector for Bacteria, Insect cells and Mammalian cells

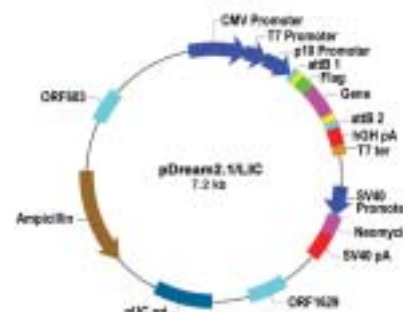
pDream2.1/LIC vector is a protein expression vector for both efficient cloning and high-level expression of any target genes. The gene of interest can be efficiently cloned into the vector using Ligation Independent Cloning (LIC) method, and can be expressed directly without any further cloning work in any one of the three major protein expression systems: Bacteria, Insect cells and Mammalian cells. The LIC cloning kit is included in the pDream2.1/LIC vector package.

Description	Cat.#	Qty
pDream2.1/LIC Kit	BM1340	1 kit

Contains : Predigested pDream2.1/LIC 1 µg (20 µl), T4 DNA Polymerase, 5X T4 DNA Polymerase Buffer, cGFP Control Insert, dATP, EDTA

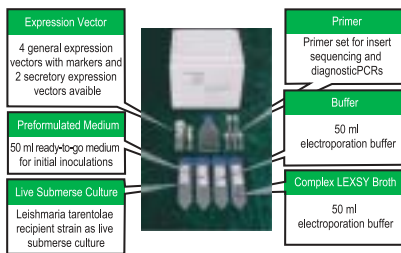
Related product
Thermus strain (HB27 :: nar)

Related product
Thermus strain (HB27 :: nar)



Eukaryotic Expression System LEXSY

The *Leishmania* Expression System (**LEXSY**) represents the combination of easy handling known from bacterial expression systems with the potential of a eukaryotic protein expression/folding/modification system.



- ◆ Rapid growth
 - Cultivation in cost-efficient media at 26°C, doubling time 4 h⁽¹⁾
 - Serum-free standard media or fully synthetic media
 - Cell densities in suspension cultures >10⁸ cells/ml
- ◆ Non-pathogenic to humans, lizard-parasite
- ◆ Received biosafety S1-clearance
- ◆ Full eukaryotic protein folding machinery
- ◆ Mammalian-type post-translational protein modifications

(1) In agitated cultures, approx. 7 h in static cultures

We offer three types of Expression Starter Kits, containing all essential components to engineer your *Leishmania* expression strain. The kits are available with

- ◆ General expression vectors (IL1570 to IL1520),
- ◆ Secretory expression vectors (IL1430 to IL2400),
- ◆ Inducible expression vectors (IL1540 & IL2140).

They include the respective antibiotic for selection of recombinant *Leishmania* expression strains.

Leishmania tarentolae Gene Expression Starter Kits - Constitutive Expression Kits

Description	Cat.#	Qty
Constitutive LEXSY Starter Kit bleomycin selection (contains vector pF4X1.4ble)	IL1570	1 Kit
Constitutive LEXSY Starter Kit hygromycin selection (contains vector pF4X1.4hyg)	IL1090	1 Kit
Constitutive LEXSY Starter Kit neomycin selection (contains vector pF4X1.4neo)	IL1530	1 Kit
Constitutive LEXSY Starter Kit nourseothricin selection (contains vector pF4X1.4sat)	IL1520	1 Kit

Each kit contains :

- *Leishmania tarentolae* laboratory strain P10 as live suspension culture
- 50 ml Complex LEXSY Broth - Complete for initial inoculations
- Components for preparation of 1 L Complex LEXSY Broth
- Expression vector with ble marker (LEXSY Bleo selection), hyg marker (LEXSY Hygro selection), neo marker (LEXSY Neo selection) or sat marker (LEXSY NTC - Nourseothricin selection)
- The respective antibiotic for 1 L medium
- 50 ml electroporation buffer (HEB)
- Primer sets for insert sequencing and diagnosis PCR
- User manual

LEXSY Kits - *Leishmania tarentolae* Cultivation Starter Kits

The *Leishmania tarentolae* Cultivation Starter Kit is the easiest way to establish a *Leishmania tarentolae* cell culture in your own laboratory. The kit contains everything to start your work with *Leishmania tarentolae* (strain as live suspension culture, media, cell culture flasks and cryo vials with glycerol).

Description	Cat.#	Qty
LEXSY Cultivation Starter Kit P10 (contains laboratory strain P10; use for constitutive and secretory expression vectors)	IL1590	1 Kit
LEXSY Cultivation Starter Kit T7-TR (contains T7-TR strain expressing bacteriophage T7 RNA polymerase and TET repressor; use for inducible expression vectors)	IL1220	1 Kit

Related products :

LEXSY Constitutive Expression Vector pF4X1.4ble (bleomycin selection)	FQ3601	5 µg
LEXSY Constitutive Expression Vector pF4X1.4hyg (hygromycin selection)	FQ3611	5 µg
LEXSY Constitutive Expression Vector pF4X1.4neo (neomycin selection)	FQ3621	5 µg
LEXSY Constitutive Expression Vector pF4X1.4sat (nourseothricin selection)	FQ3631	5 µg
LEXSY Secretory Expression Vector pF4SPepoX1.4sat (nourseothricin selection)	IL1240	5 µg
LEXSY Secretory Expression Vector pF4SPImsapX1.4sat (nourseothricin selection)	IL1390	5 µg
LEXSY Secretory Expression Vector pF4SPImsapX1.4hyg (hygromycin selection)	IL1380	5 µg
LEXSY Secretory Expression Vector pF4SPImsapX1.4neo (neomycin selection)	IL1810	5 µg
LEXSY Inducible Expression Vector pTUBAPX1.4ble (bleomycin selection)	IL1370	5 µg
LEXSY Inducible Expression Vector pTUBAPX1.4neo (neomycin selection)	IL1340	5 µg

Leishmania tarentolae Gene Expression Starter Kits - Secretory Expression Kits :

Secretory LEXSY Starter Kit EPO signal peptide nourseothricin selection (contains vector pF4SPepoX1.4sat)	IL1430	1 Kit
Secretory LEXSY Starter Kit LMSAP signal peptide nourseothricin selection (contains vector pF4SPImsapX1.4sat)	IL2090	1 Kit
Secretory LEXSY Starter Kit LMSAP signal peptide hygromycin selection (contains vector pF4SPImsapX1.4hyg)	IL2450	1 Kit
Secretory LEXSY Starter Kit LMSAP signal peptide neomycin selection (contains vector pF4SPImsapX1.4neo)	IL2400	1 Kit

Each kit contains :

Leishmania tarentolae laboratory strain P10 as live suspension culture

- 50 ml Complex LEXSY Broth - Complete for initial inoculations
- Components for preparation of 1 L Complex LEXSY Broth
- Expression vector with sat marker (LEXSY NTC - Nourseothricin selection)
- The respective antibiotic for 1 L medium
- 50 ml electroporation buffer (HEB)
- Primer sets for insert sequencing and diagnosis PCR
- User manual

LEXSY can be used without a license in any not-for-profit research in academic or public institutions.

The use of the Leishmania Expression System (LEXSY) and its components for all commercial purposes however, requires a separate license. Commercial use includes but is not limited to :

- ◆ The use of any protein or other substance produced by the LEXSY System as reagents in screening to discover and/or promote candidate compounds for sale to a customer, distributor, wholesaler or other end user in therapeutic, diagnosis, prophylactic, and/or veterinary areas,
- ◆ The manufacture, sale or offer to sell of any product containing proteins or other substances produced by the Expression System,
- ◆ The large scale production of pharmaceuticals recombinant protein
- ◆ "Contract research" to any third party or "Contract manufacturing" for any third party that has not been granted by a license to use LEXSY.

Leishmania tarentolae Gene Expression Starter Kits - Inducible Expression Kits :

Description	Cat.#	Qty
Inducible LEXSY Starter Kit1 bleomycin selection (contains vector pTUBAPX1.4ble)	IL1540	1 Kit
Inducible LEXSY Starter Kit2 neomycin selection (contains vector pTUBAPX1.4neo)	IL2140	1 Kit

Each kit contains :

Leishmania tarentolae host strain T7-TR expressing T7 RNA Polymerase and TET repressor as live suspension culture

- 50 ml Complex LEXSY Broth - Complete for initial inoculations
- Components for preparation of 1 L Complex LEXSY Broth
- Expression vector with ble marker (LEXSY Bleo selection) or neo marker (LEXSY Neo selection)
- the respective antibiotic for 1 L medium
- 50 ml electroporation buffer (HEB)
- Primer sets for insert sequencing and diagnosis PCR
- User manual

LEXSY Supplements : Expression stain and media

Description	Cat.#	Qty
L. tarentolae laboratory strain P10 for constitutive and secretory expression (Live suspension culture)	IL5200	10 ml
L. tarentolae T7-TR strain for inducible protein expression constitutively expressing bacteriophage T7 RNA polymerase and TET repressor (Live suspension culture)	IL5190	10 ml

Complex media

LEXSY Broth BHI - liquid media kit (brain heart infusion based) recommended for transfection and strain maintenance	IL1050 IL1051 IL1052	1 L 5 L 20 L
LEXSY Broth BHI - powder media kit (brain heart infusion based) Recommended for transfection and strain maintenance	IL1200 IL1201 IL1202	1 L 50 L 10 L
LEXSY Broth YC - liquid media kit (yeast-casein based) Recommended for high cell density fermentation	IL1290 IL1291 IL1292	1 L 5 L 5 L
LEXSY Broth YC - powder media kit (yeast-casein based) Recommended for high cell density fermentation	IL1800 IL1801 IL1802	1 L 5 L 10 L
LEXSY Broth YS - liquid media kit (yeast-soy based) casein-free Recommended for high cell density fermentation	IL1700 IL1701 IL1702	1 L 5 L 10 L
LEXSY Broth YS - powder media kit (yeast-soy based) casein-free Recommended for high cell density fermentation	IL1140 IL1141 IL1142	1 L 5 L 10 L

Please note :

- ◆ Additives like Hemin and Pen-Strep are **required in any case** for optimal growth of *Leishmania* cultures
- ◆ Addition of these components to the media will decrease the time interval during which these media are usable - for special instructions please consult the particular media datasheets
- ◆ If media are used after the indicated expiry date, additives have to be added again in appropriate amounts.

Synthetic (serum-free) media

Description	Cat.#	Qty
Synthetic LEXSY Broth, liquid, ready-to-go (sterile, contains Hemin and Pen-Strep) shelf live 2 weeks	IL1720	1 L
	IL1721	5 L
	IL1722	10 L
Synthetic LEXSY Broth - liquid media kit (sterile, contains Hemin and Pen-Strep stock solutions) shelf live 6 month	IL1750	1 L

Additives

Hemin (porcine) sterile 500x stock solution in 50% triethanolamine (for 1/5/10 L medium)	OL2460	2 ml
	IL2461	10 ml
	IL2462	20 ml
Pen-Strep sterile 200x stock solution of penicillin and streptomycin (for 1/5/10 L medium)	IL1770	5 ml
	IL1771	25 ml
	IL1772	50 ml

Silencing

TransIT-TKO® siRNA Transfection Reagent

- ◆ Designed specifically for high efficiency siRNA delivery in mammalian cells.
- ◆ Low toxicity reagent proven to work effectively in a wide range of cell types.
- ◆ Results in silencing of greater than 95% of target gene expression.
- ◆ One step, serum compatible reagent supplied with an optimized protocol

Cellular up-take of long double stranded RNA (dsRNA) has been shown to induce RNA interference in a diverse group of organisms as well as insect cells in culture. RNA interference leads to the inhibition of protein expression by utilizing sequence-specific, dsRNA-mediated destruction of the target messenger RNA (mRNA). Attempts to induce RNA interference using long dsRNA in mammalian cell lines have been met with limited success, due in part to the induction of the interferon response, which results in a general inhibition of protein synthesis. Recently, it has been shown that when short RNA duplexes are introduced into mammalian cells in culture, sequence-specific inhibition of target mRNA can be realized without introducing an interferon response. These short dsRNA, referred to as small interfering RNAs (siRNA), act catalytically at sub-molar ratios to cleave greater than 95% of the target mRNA in the cell. The RNA interference effect can be long-lasting and may be detectable after many cell divisions. These properties make siRNA extremely effective at inhibiting target gene expression once introduced into the cell.

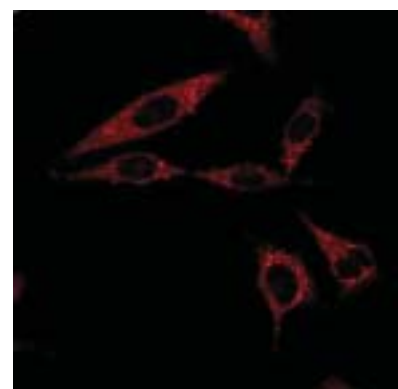
In recognition of these significant findings, **TransIT-TKO® Transfection Reagent** has been developed, which enables highly efficient siRNA transfection with significantly reduced levels of cell damage as compared to cationic-liposome based transfection reagents. Transfections are most effective when carried out in complete growth media, with no media change or serum addition required. TransIT-TKO® Reagent, when complexed with siRNA, knocks down target gene expression in a variety of cell lines. These unique features make TransIT-TKO® Transfection Reagent ideal for all siRNA-mediated gene silencing studies.

Cell Lines Successfully Transfected :

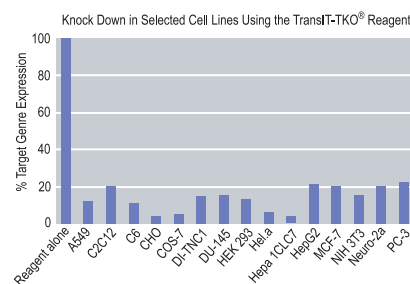
A549, BNL.CL2, C2C12, C6, CHO-K1, COS-7, Daoy, DB-TRG-05MG, D1-TNC1, DU145, HEK 293, HeLa, Hepa 1-6, Hepa1cLc7, Hep G2, human astrocytes, Jurkat, Keratinocytes (NIKS), MCF-7, Neuro-2a, NIH 3T3, PC-3, primary mouse hepatocytes, primary rat hepatocytes, RAW 264.7, SK-N-MC, THP-1, Vero.

Description	Cat.#	Qty
TransIT-TKO® Transfection Reagent	T37400	1 ml
	T37401	0.4 ml
	T37403	5 x 1 ml
	T37407	10 x 1 ml

T37400 provides sufficient reagent to perform up to 1000 transfections in 24-well plates.



HeLa cells transfected with LabelIT® siRNA Tracker™ Biotin labeled siRNA and TransIT-TKO® Transfection Reagent in complete media in 24 hours, fixed then stained with Cy^{5.3} Streptavidin.



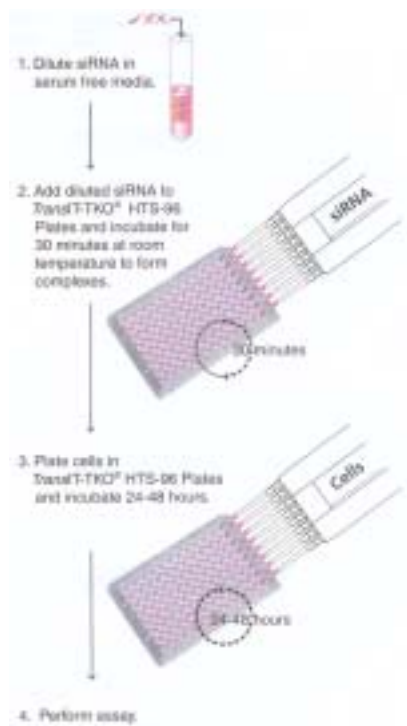
Reporter plasmids expressing firefly and sea pansy luciferase were co-transfected into a variety of cells using *TransIT-LT1®* transfection reagent. Firefly luciferase expression was knocked down using anti-firefly luciferase siRNA complexed with *TransIT-TKO®* transfection reagent. Bars indicate the percent firefly luciferase expression as compared to the untargeted sea pansy luciferase 24 hours after delivery of 5 nM anti-firefly luciferase siRNA with *TransIT-TKO®* Reagent.

Related Products :

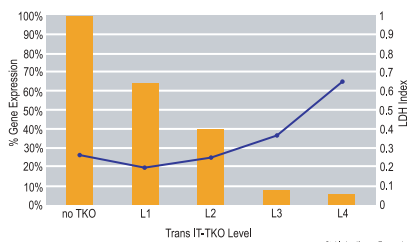
Label IT® siRNA Tracker Kits with TransIT-TKO® Transfection Reagent
TransIT®-LT1 and *-LT2* Transfection Reagents (Product # T06361, J29041)
 MiraCLEAN™ Endotoxin Removal Kit (Product #5900)

Ask us the bibliography about these products.

Protein expression study



Comparison of Knockdown Efficiency and cellular Viability Using TransIT-TKO HTS-96 Titration Plates in 3T3-Lux Cells



NIH3T3 cells stably transfected with a plasmid encoding firefly luciferase (Lux) were screened in a TransIT-TKO HTS-96 Titration Plate (four reagent levels) using 5 nM of an siRNA targeting firefly luciferase. Results correlate efficiency of firefly luciferase knockdown and toxicity measured by LDH release. The data indicates an optimal at Level 2 to 3, Level 2 achieves 60% gene knockdown with no change in cell viability, and Level 3 achieves 93% gene knockdown with only a moderate increase in LDH released as compared to a cells alone control.

TransIT-TKO HTS-96 plates

Features

- ◆ Ideal for screening applications : High efficiency knockdown in a 96-well plate format allows rapid, simultaneous screening of multiple siRNA conditions including siRNA concentration, sequence and cell type in one plate.
- ◆ Customized : Plates are available in four working concentrations of TransIT-TKO Reagent for optimization with a wide range of cell types. The Titration Plate is available for testing all four levels at one time in a preliminary screen.
- ◆ Convenient : *Easy-to-use*, serum compatible protocol; can be performed in a single 96-well plate or using a *two-step* transfer method.
- ◆ Consistent performance : Efficient and reproducible siRNA delivery among multiple wells in a plate for performing replicate samples.

TransIT-TKO HTS-96 Plates provides high efficiency siRNA transfection reagent in a 96 well plate format suitable for screening applications such as target validation. Increase the speed of testing multiple siRNA sequences and concentrations and quickly determine the optimized conditions to achieve high efficiency *knock-down* in a specific cell type. The *TransIT-TKO* Transfection Reagent comes supplied as a dry film in each well of a clear, sterile 96 well plate suitable for cell culture. The plates are available in four different working concentrations of *TransIT-TKO* Reagent since different cell types require a specific amount of reagent for optimized delivery and *knock-down*. This *easy-to-use* format is adaptable to high volume processing. Simply resuspend the transfection reagent with the siRNA and incubate a short time before adding the cells to the wells. Transfections are most effective when carried out in complete growth media, with no media change. *TransIT-TKO* effectively delivers siRNA targeted against both transient transfected and stable genes in a variety of cell lines, resulting in *knock-down* levels as high as 95% for a specific gene.

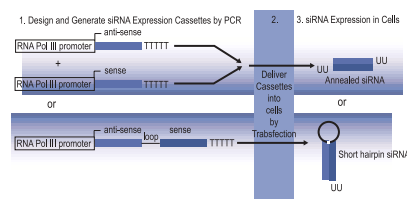
Description	Cat.#	Qty
TransIT-TKO HTS-96 Titration Plate	AM7180	1 plate ⁽¹⁾
TransIT-TKO HTS-96 Level 1 Plate	AM7190	1 plate
	AM7191	5 plates
TransIT-TKO HTS-96 Level 2 Plate	AM7200	1 plate
	AM7201	5 plates
TransIT-TKO HTS-96 Level 3 Plate	AM7210	1 plate
	AM7211	5 plates
TransIT-TKO HTS-96 Level 4 Plate	AM7220	1 plate
	AM7221	5 plates

⁽¹⁾ each titration plate contains 3 columns (24 wells) of each of the four levels of TransIT-TKO Reagent.

siXpress PCR Vector Systems

- ◆ Enables efficient in situ expression of siRNA and *knock-down*.
- ◆ Synthesize multiple siRNA expression cassettes by PCR in hours.
- ◆ Select the siRNA expression cassette that provides the most desirable effect.
- ◆ Option of cloning PCR generated expression cassette in supplied vector.
- ◆ Complete Systems : provided with primers, template, controls and TransIT-LT1 Transfection Reagent.

The **siXpress PCR Vector Systems** contain reagents required for the optimized preparation and transfection of siRNA expression cassettes. Three siXpress PCR Vector Systems are available, each utilizing a different polymerase III promoter: human U6, mouse U6, and human H1 (individual cell lines may vary in their promoter preferences). Double-stranded DNA containing the siRNA expression cassette is generated by polymerase chain reaction (PCR) and transfected in vitro into mammalian cells using TransIT-LT1 Transfection Reagent. Following nuclear transcription of the siRNA expression cassette, siRNA transcripts are exported into the cytoplasm and enter the RNAi pathway. RNAi applications include verification of effective siRNA sequences, studies of gene function, drug development, target validation, and other biological studies. The optimal PCR-generated expression cassettes can also be cloned into the supplied Template/Cloning Vector and grown under kanamycin selection in bacteria to provide a large amount of plasmid for further gene silencing experiments.



Each siXpress® PCR Vector System provides sufficient materials to generate 20 siRNA expression cassettes. The supplied Luciferase Control Vector generates a siRNA hairpin directed against the Firefly Luciferase (*luc+*) gene (Promega). PCR products generated from this vector can be used as negative controls in experiments with user-designed siRNA expression cassettes. The Control Vector can also be used as a positive control in cells transiently or stably expressing luciferase. Each system is supplied with a promoter-specific template plasmid, upstream promoter primer, downstream sequencing primer, control anti-luciferase hairpin siRNA vector, downstream control primer and TransIT®-LT1 Transfection Reagent.

Description	Cat.#	Qty
Human U6 Promoter Vector Systems	AJ1210	20 reactions
Human H1 Promoter Vector Systems	AJ1220	20 reactions
Mouse U6 Promoter Vector Systems	AJ1230	20 reactions

siXpress™ PCR Vector Systems Components

pMIR-hU6, human U6 Template/Vector	A00940	5 µg
pMIR-hU6-Luc, human U6 Luciferase Vector	A00930	5 µg
pMIR-hH1, human H1 Template/Vector	A00960	5 µg
pMIR-hH1-Luc, human H1 Luciferase Vector	A00950	5 µg
pMIR-mU6, mouse U6 Template/Vector	A00970	5 µg
pMIR-mU6-Luc, mouse U6 Luciferase Vector	A00980	5 µg
Upstream Promoter Primer	A01010	50 reactions
Downstream Sequencing Primer	A01000	50 reactions
Control Downstream Primer	BL9930	50 reactions

Plasmidic siRNA Expression Vectors

These vectors are designed for mammalian transfection. They carry a Neomycin, Hygromycin, or Zeomycin resistance gene as selectable marker, which can be used for establishing stable cell lines. The pRNAT vectors carry a GFP marker (coral GFP, cGFP) under CMV promoter control, which can be used to track transfection efficiency. They use U6 (regular or enhanced), H1 (regular or enhanced), or CMV promoter to drive the siRNA expression.

siRNA Expression Vector pRNAT-H1.1/Neo

Description : pRNAT-H1.1/Neo is a Genscript siRNA expression vector. It is designed for mammalian transfection. It carries a Neomycin resistance gene as the selectable marker which can be used for establishing stable cell lines. The GFP marker (coral GFP, cGFP) under CMV promoter control can be used to track the transfection efficiency. It uses H1 promoter for siRNA expression.

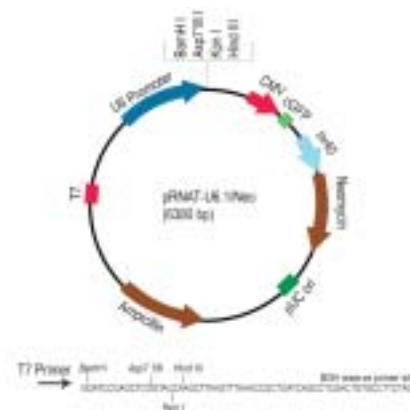
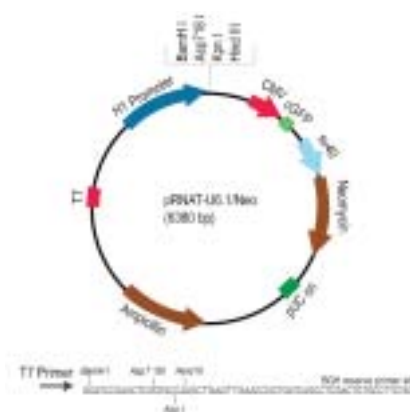
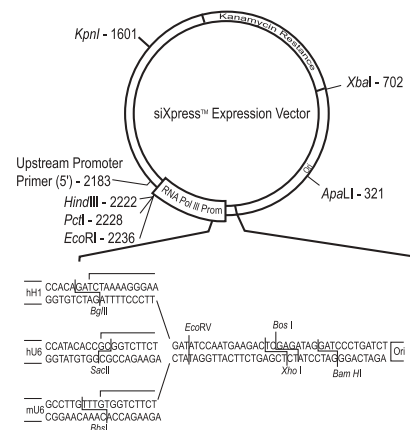
Description	Cat.#	Qty
pRNAT-H1.1/Neo	AP2810	10 µg

siRNA Expression Vector pRNAT-U6.1/Neo

Description : pRNAT-U6.1/Neo is a Genscript siRNA expression vector. It is designed for mammalian transfection. It carries a Neomycin resistance gene as the selectable marker, which can be used for establishing stable cell lines. The GFP marker (coral GFP, cGFP) under CMV promoter control can be used to track the transfection efficiency. It uses U6 promoter for siRNA expression.

Description	Cat.#	Qty
pRNAT-U6.1/Neo	AP2830	10 µg

To optimize the experimental parameters of DNA vector-based siRNA constructs, we have designed positive controls which can *knock-down* Firefly Luciferase and Renilla Luciferase, which you can use for your initial test experiment.



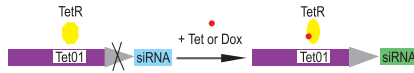
Biochemistry & Molecular Biology Analysis

Protein expression study

For other plasmid, please contact us at interbiotech@interchim.com

Inducible siRNA Expression Vectors

The H1.2 promoter is an engineered inducible promoter containing a tetracycline operator (TetO1). The Tetracycline operator itself has no effect on expression. When the tetracycline repressor (TetR) is present. It effectively binds the TetO1 and blocks the transcription. In the presence of tetracycline or doxycycline, the inducer binds TetR, causes the TetR protein to release the TetO1 site, and derepresses the transcription from the H1 promoter.

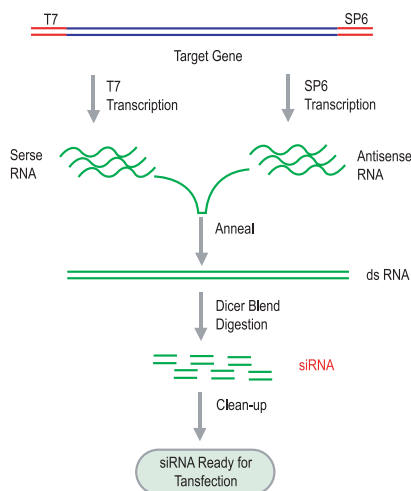


pRNATin-H1.2 is designed for mammalian transfection. They carry a Neomycin or hygromycin resistance gene which can be used for establishing stable cell line and a GFP (coral GFP) marker under CMV promoter* control to track the transfection efficiency. The adenoviral, retroviral, and lentiviral expression vectors are also available for this inducible system.

Name	Promoter	Resistance	Marker	Cat.#
pRNATin-H1.2/Neo	H1.2	Neo	cGFP	BM1510
pRNATin-H1.2/Hygro	H1.2	Hygro	cGFP	BM1520
pRNAin-H1.2/Neo	H1.2	Neo	-	BM1530
pRNATin-H1.2/Adeno	H1.2	Kanamycin	cGFP	BM1540
pRNAin-H1.2/Shuttle	H1.2	Neomycin	-	BM1550
pRNATin-H1.2/Retro	H1.2	Neo	cGFP	BM1560
pRNATin-H1.2/Lenti	H1.2	Neo	cGFP	BM1570

Knock-down siRNA Kits

- ◆ Flexible : generate siRNA in your own laboratory.
- ◆ Efficient : because this kit starts with a DNA fragment of the target gene, it can generate a mixture of siRNAs that has much more chance to knock-down the target gene than a single or few siRNAs.
- ◆ Cost Effective : one kit can be used for five genes knock-down experiments and more than 100 transfections.
- ◆ Enzyme digestion needs only 15 minutes



Small interfering RNAs (siRNAs) are double stranded RNAs that the mediators of mRNA degradation in the process of RNA interference (RNAi). RNAi has been used as a tool to study gene functions. The siRNA appears that longer dsRNA is processed into 21-23 nt dsRNA (siRNA) by an enzyme, Dicer. Knock-down siRNA Kit provides everything you need for siRNAs generation from the DNA fragment of target genes. Customers who have the constructs of target genes in vectors containing any two of the promoters, T7, T3, and/or SP6 at the 5'-end and 3'-end of inserted gene can directly generate siRNAs with this kit within one day.

References :

- Sharp P. A. (2001) *Genes Dev.* 15, 485 - 490.
 Bernstein, E., et al. (2001) *Nature* 409, 363 - 366.
 Knight S. W., et al. (2001) *Science* 2, 2269-2271.

B.222 Knock-down siRNA Kit Procedure.

Product	Sub Type	Cat.#	Qty
Knock-down siRNA Kit 1	T7 Transcription Kit siRNA Kit	AM6950	5 rxns
Knock-down siRNA Kit 2	SP6 Transcription Kit siRNA Kit	AM6970	5 rxns
Knock-down siRNA Kit 3	T3 Transcription Kit siRNA Kit	AM6990	5 rxns
Knock-down siRNA Kit 4	T7 Transcription Kit siRNA Kit	AM7020	5 rxns
Knock-down siRNA Kit 5	T7 Transcription Kit siRNA Kit	AM7030	5 rxns
Knock-down siRNA Kit 6	SP6 Transcription Kit siRNA Kit	AM7040	5 rxns
siRNA Kit	Dicer Blend etc.	AM7050	5 rxns

See also transfection of nucleic acids pages D103-D109