Proteomics - Protein Expression

Protein Expression & Production

Technical tip – **Protein expression :**
Transfection / Recombinant protein production / Protein reporting / Protein Silencing

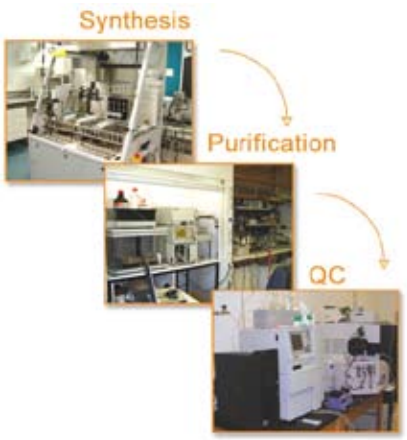
Many technologies were proposed to induce **protein expression** in cell lines, and answer the needs of proteomics research (study of **protein levels**, **localization**, **regulation**,...) to large scale production in biotechs (**production** of vaccine, hormones,...). Genetic sequence (gene, or cDNA) of foreign proteins can be transferred into cells by electroporation or through engineered vectors (**transfection**). A variety of vectors and cell lines achieve particular efficiency to express protein, i.e. at low to high level, toxic proteins, soluble proteins, rapidly (for HTS)... Most use prokariotic cells (i.e. *E.coli*) or yeast (i.e. *Saccharomyces cerevisiae*), which are not equipped with full machinery to modify proteins as in superior organisms. Systems using Insect were introduced more recently. Lastly, CHO cells, HEK-293 cells or other mammalian cell lines, that are equipped with machinery, can be transfected, but are usually not suited for flexible protein expression nor for protein production in large scale.

Expression systems co-expressing a marker (GFP, β-galactosidase, Aquaeporin...) were popularized for **reporter assays**, achieving sensitive detection of a target protein in cell cultures, tissues, or in-vivo. Additionally, an other alternative for protein expression study as risen : Protein expression **silencing** has become a valuable method for the study of the expression and activity of a protein in cells (siRNA, microRNA). Unique information is obtained to show that 1/the observed effect is due to the protein itself, and not from indirect effects 2/ the post traductional modifications are responsible of the observed effect , ...

Interchim offers great and innovative solutions to express, purify and characterize proteins that mach your exact needs.

BioSciences Innovations is pleased to present here :

- custom production of **phospho-specific antibodies** against phosphorylated proteins,
- **protein expression/production systems**, including 2 remarkable methods related to functional proteomics research, as the Lexsy expression system (with full eukaryotic protein expression/ folding/ modification) and the pLive system for long duration expression,
- **Protein expression silencing by siRNA**, including the production of siRNA by DNA-vector technology (siXpress), and TKO transfection agent,
- **protein expression or silencing by microRNA** for post-transcriptional gene expression studies,
- **Protein reporter assays**, including our superior Luciferase assays,and Renilla mulleiri GFP vectors.



Peptide synthesis

Custom peptide synthesis is available for native or modified peptides :

Phosphorylation	Enzyme labeled peptides
Glycopeptides	Fluorescent labeled peptides
FRET labeled peptides	Unusual amino-acids containing peptides
Cyclic peptides	Sulfated, methylated or fluorinated peptides
MAPS peptides	Single or multiple phosphorylated peptides
Pegylated peptides	Long peptides (>100 aa !)
Biotinylated peptides	

Please ask **interbiotech@interchim.com** for a quote providing :

. sequence,
. eventual modifications,
. required quantity.

The peptide is synthetized, purified and sent to you along with QC documents.

LEXSY protein expression system

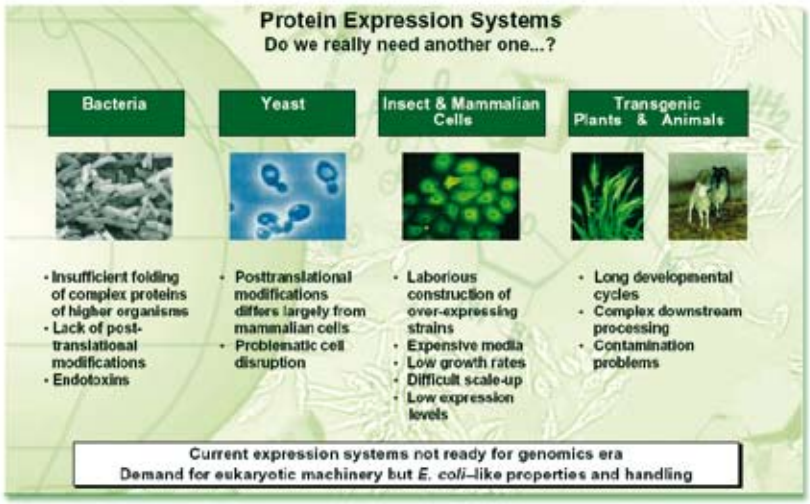
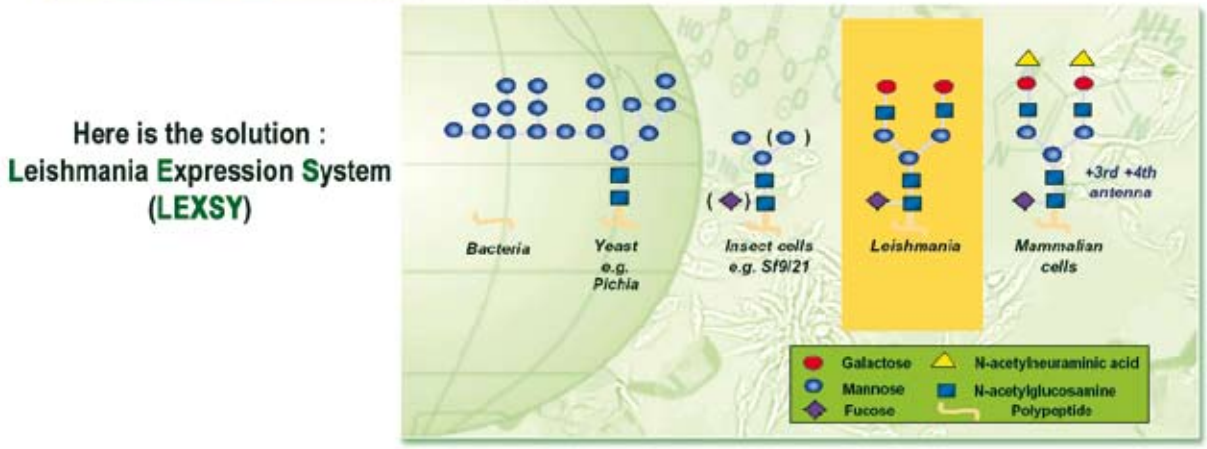


Figure 1



- **Full Eucaryotic protein machinery**
- **Mammalian-type post-translational protein modifications**
- **High protein expression level (>0.1mg/ml)**
- **4 markers for co-expression of complex proteins (oligomers)**
- **vectors designed for constitutive, secretory or inducible expression**
- **Rapid growth :**
  - Cultivation in cost-efficient media at 26°C / doubling time 4H
  - Cell densities in suspension culture >108 cells/ml

LEXSY (**L**eishmania **E**xpression **S**ystem) uses *Leishmania tarentolae*, a protozoaire micro-organism that is, as Escherichia coli, non pathogenic (received biosafety S1-clearance). One of the most intriguing features of LEXSY is its **mammalian-like glycosylation resulting in favorable expression and activity data** especially for glyoproteins including examples such as homogenously glycosylated human recombinant erythropoietin (EPO), human transcription factors, and human interferon gamma expressed as the 2 forms known from human preparations. Additionnaly to this potential of a eukaryotic protein expression/folding/modification system (without the limitations of insect, mammalian or plant or tissue based methods), LEXSY system combines the advantages of bacteria and yeast organisms that are robust systems for large scale and rapid protein production (fig.1). It uses economic selection and culture media. Yield over >0.1mg/L were obtained with >76% of expressed proteins.

Protein Expression & Production

Hence, LEXSY expression system is a a great solution for proteomics, from research to application level. Expression "drafts" can be generated in *E.coli*. The target gene can be introduced into *L. tarentolae* on a circular plasmid, that is maintained in *L. tarentolae* as episomes transiently without selection or stable with selection (can be integrated into the Leishmania genome upon successive cultivation for higher expression). After transfection, selection of recombinant cell lines, and clonage, cells express the protein. It was shown that essentially all stable transfected cells express the expression cassette. Lastly, LEXSY has been used in in-vivo protein labeling for structure research (^{15}N-HSQC NMR).

List of references available on inquire.

The new **LEXSYcon2 Expression Kit** is the first choice for expression. The new, versatile constitutive pLEXSY-2 vector permit highly efficient expression of most proteins. Dependent upon cloning strategy the vectors **allow cytosolic expression of standard proteins or secretory expression of proteins that undergo post-translational modifications** (PTMs) such as glycosylation or disulfide bond formation. In this case efficient export of target proteins is achieved by fusion to a Leishmania export signal peptide. It provides more flexibility in cloning, combining both features of previous LEXSYcon Kit for constitutive expression and LEXSecrete Kit for secretory expression.

The new **LEXSinduce2 Expression Kit** is recommended **for proteins that may exert toxic effects upon cells**. Expression is efficiently regulated by the T7-TR architecture with very low background, and protein production is induced by addition of tetracycline (pLEXSY_I2 vector).The new LEXSinduce2 Kit replaces the previous LEXSinduce Kit for constitutive expression and **allows cytosolic or secretory expression**.

	Starter Kit cat. #, Qty	Vectors cat. #, Qty
LEXSYcon2 Expression Kit for constitutive cytosolic or secretory expression The kit contains : . pLEXSY-sat2 (nourseothricin selection) [a] . LEXSY host strain P10 as glycerol stock . primer sets for insert sequencing and diagnostic PCR . all components for preparation of 1 L LEXSY BHI medium . antibiotic for 1 L medium	**CP9730**, 1 Kit	**CP7650**, 5 µg
LEXSinduce2 Expression Kit for inducible expression The kit contains : . pLEXSY_I-neo2 (neomycin selection) [a] . LEXSY host strain T4-TR as glycerol stock . primer sets for insert sequencing and diagnostic PCR . all components for preparation of 1 L LEXSY BHI medium . antibiotic for 1 L medium	**CV3580**, 1 Kit	**CQ7470**, 5 µg

[a] both kits/vectors are available as well with 4 marqueurs for bleomycyn, hydromycin, neomycin, nourseothricin)

Note : LEXSY system can be used for research and development, but its use for any commercial purposes requires a separate license from Jena Bioscience

Please inquire for single components, cultivation starter kits, media and additives, antibiotics, cloning vectors, and tools electrophoration buffer and cell culture flask (or see catalog BioSciences Innovations pages B216-B219).

Protein expression & reporting

Firefly Luciferase 1-Step Assay Kit, 2 h reading

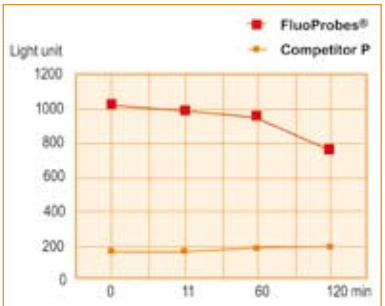
- **Reproducibility** – CV less than 5%
- **Linear range**- Assay linear over seven orders of magnitude
- **Limit of detection** – less than 1 fg of luciferase per sample
- **No disposal problems or hazards** are associated with the use of these luciferase assay kits.

FluoProbes' Luciferase 1-Step assay system is a homogeneous high sensitivity firefly luciferase reporter gene assay kit with a half-life of 2 hours for the quantification of firefly luciferase expression in mammalian cells. This kit is specially designed for batch processing systems using microplates such as 96-well plates. FluoProbes' Luciferase 1-Step assay kit offers higher sensitivity and wider dynamic range for detecting luciferase activity within mammalian cells (figure), consistent reproducibility and cost effectiveness along with the added convenience of a one step assay.

Name	Cat#	Qty
Firefly Luciferase 1-Step Assay Kit	**FP-BX0320**	100 ml (1000 tests in 96-well plate)
Firefly Luciferase 1-Step Assay Kit	**FP-BX0321**	1000 ml (10000 tests in 96-well plate)

Related available products :
Other Luciferases and Luciferases kits (Firefly, Renilla, Gaussia)
A large choice of luciferines and coelenterazines substrates (pages E49-E51 of the BioScience catalogue), including :

Name	Cat#	Qty
D-Luciferin K + salt	**FP-M1224D**	1 g
D-Luciferin free acid	**FP-27060A**	100 mg
	FP-27060D	1 g

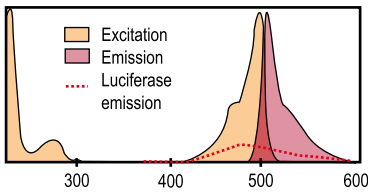


Comparison of Steady Glow Kinetics between FluoProbes' 1-Step kit and competitor kit.
This kit has a special additive incorporated into the reagent which stabilizes the light emission. However, the stabilizer we use is not an inhibitor of the luciferase enzyme. Therefore this kit enables measurement of luciferase activity over an extended time period without compromising the levels of luciferase expression. (Data is presented as mean of quadruplicate determinations, CV less than 5%)

Reporter systems : GFP & lacZ

Renilla GFP is a bright popular Green Fluorescent Protein. We provide inovative GFP vectors. lacZ is the most popular reporter enzyme used in genomics and proteomics. We provide lacZ assay kits.
Please inquire for other reporter systems (SEAP,...).

Renilla reniformis



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.

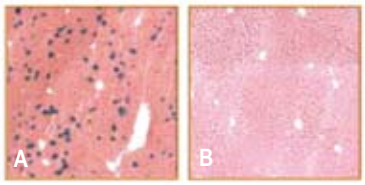
Renilla GFP vectors

- Renilla GFPs are over 3 times brighter than Aequaporin eGFP
- Almost symmetrical excitation and emission peaks
- Expression in bacteria, fungi and mammalian cells (Hela, Cos1 and CHO)

Name	Cat#	Qty
Renilla mullerei native GFP in pUC19 backbone for expression in bacterial cells	**FP-AM6840**	25 µg
Renilla reniformis native GFP in pUC18 backbone for expression in bacterial cells	**FP-AM6850**	25 µg
Renilla mullerei humanized GFP in pGEX-4T backbone for expression in mammalian cells, also express well in fungi.	**FP-BL8680**	25 µg

ß-Galactosidase Staining Kits

One of the most common reporter genes used in molecular biology applications is the *E. coli* lacZ gene that codes for an active subunit of ß-galactosidase in vivo, because it is absent in normal mammalian, yeast and even plants cells.



Strong Liver Expression of ß-galactosidase from the pLIVE™-lacZ Vector after Hydrodynamic Tail Vein Injection.The pLIVE™-lacZ Vector (Panel A) was delivered to an ICR mouse using the hydrodynamic tail vein injection procedure and the TransIT®-QR Hydrodynamic Delivery Solution (BM4530). At 24 hours post-injection, the liver was harvested, sectioned and stained with X-gal using the Beta-Gal Staining Kit (J29660) to demonstrate ß-galactosidase activity (blue cells). The cells were then counterstained with hematoxylin and eosin Y to stain the nuclei and cytoplasms, respectively. The control mouse (lacZ negative) in Panel B was stained in parallel to Panel A and contains no detectable ß-galactosidase activity.

Name	Cat#	Qty
ß-Galactosidase Staining Kit, Colorimetric blue staining Contains sufficient reagents for stainings 150 assays in 35 mm wells (Cell Fixative Reagent, Cell Staining Solution and X-GAL Reagent, buffer).	**BN6881**	1 kit
FACS Blue lacZ ß-Galactosidase Detection Kit, Fluorogenic blue staining Contains : 0.5 ml substrate solution (4x), reference standard , 1 ml inhibitor	**FP-BM8410**	10 x 96 tests
In vivo lacZ ß-Galactosidase Intracellular Detection Kit Contains : Fluorescent Substrate, Reference standard, Inhibitors (chloroquinine, PETG)	**FP-BN7260**	1 kit
lacZ ß-Galactosidase Detection Kit - Chemiluminescent Contains all the necessary reagent, buffers, substrate (chemiluminescent 1,2 Dioxetane substrates, which emit visible light), and protocols for sensitive and quantitative lacZ ß-galactosidase activity assay in mammalian, yeast, and bacterial cells.	**FP-BM8420**	1 kit

See detailed description page D72 of BioScience Innovation catalogue, or inquire at interbiotech@interchim.com.

Related products: ß-Galactosidase substates

Name	Cat#	Qty
IPTG (inducer of β-Galactosidase)	**UP84853C**	1 g
X-Gal (blue)	**UP40534M**	1 g
Green-β-D-Gal, MW : 309.3, green	**AM338A**	25 mg
Rose-β-D-Gal ,MW : 329.74, pink (~540 nm)	**AM341A**	100 mg
Red-β-D-Gal, MW : 408.6, red/magenta (~565 nm)	**A27020**	100 mg
Purple-β-D-Gal, MW : 421.19, purple (~575 nm)	**AM339A**	25 mg
oNPG, Yellow (410nm)	**UP556683**	5 g
MU galactopyranoside	**UP248742**	1 g
Fluorescein di-β-D-Galactopyranoside (FDG), Green (490/514nm)	**FP-52476A**	5 mg
Fluorescein mono-β-D-Galactopyranoside (FM-Gal), Green (490/514nm)	**FP-524771**	5 mg
Resorufin-β-D-Galactopyranoside (Res-Gal), Red (571/585)	**FP-52473A**	50 mg*
4-Methylumbellifery-β-D-Galactopyranside (MU-Gal), Blue (360/449nm)	**FP-248742**	5 g*
4-Trifluoromethylumbelliferyl-β-D-Galactopyranoside (TFMU-Gal), Aqua (385/501nm)	**FP-M1141A**	25 mg
Carboxyumbelliferyl-β-D-Galactopyranoside (CUG), Light Blue (330/448nm)	**FP-M1171A**	10 mg
3-Carboxyumbelliferyl-β-D-Galactopyranoside-BSA conjugate, Light Blue (330/448nm)	**FP-BM8390**	10 mg
β-Galactosidase Sample Kit, Red (571/585), Green (490/514), Aqua (385/501), Blue (330/448)	**FP-BM8400**	1 kit

Many other substrates of other reporter enzymes (i.e. β-glucurosidase) are as well available. See page D74-D75 of BioScience catalogue, or inquire at interbiotech@interchim.com.

Protein Expression & Silencing (siRNA, miRNA)

Protein expression silencing has become a valuable method for the study of the expression and activity of a protein in cells. Unique information is obtained to show that the observed effect is due to the protein itself, and not from indirect effects of the protein expression, i.e. if the post traductional modifications are responsible. While **siRNA** based silencing have been well popularized, research pay attention to **miRNAs** that are believed to play a crucial role in eukaryotic development by controlling post-transcriptional gene expression.

Interchim offers great tools regarding these 2 strategies :

- **vector based microRNA technology**, for analysis for post-transcriptional gene expression studies
  . miRNA vectors allowing you to construct miRNA your self;
  . miRNA construction service in the vector of your choice,
  . miRNA analysis with BioAnalyser system (Agilent).
- **transfection of siRNA** for protein silencing and knock-down
  . transfection agent UptiFectin OFF.

vector based microRNA technology (miRNA)

miRNA vectors

These vectors are designed for long-term gene silencing experiments in a broad range of mammalian cell lines. It is an offshoot of our CMV-based siRNA vectors and carries an RNA polymerase II-type CMV promoter (human cytomegalovirus immediate-early promoter) and an optimized SV40 polyadenylation signal to drive high-level expression of the hairpin RNA. This vector also features an SV40 promoter that expresses one of three antibiotic resistance genes (hygromycin, neomycin, or puromycin) for long-term antibiotic selection used for stable transfections.

miRNA vectors

Vecctor Name	Promoter	Resistance	Marker	Cat.#
pRNA-CMV3.1/Neo	CMV	Neomycin	-	**CF3590**
pRNA-CMV3.1/Hygro	CMV	Hygromycin	-	**CF3600**
pRNA-CMV3.1/Puro	CMV	Puromycin	-	**CF3610**
pRNAT-CMV3.1/Neo	CMV	Neomycin	cGFP	**CF3620**
pRNAT-CMV3.1/Hygro	CMV	Hygromycin	cGFP	**CF3630**
pRNAT-CMV3.1/Puro	CMV	Puromycin	cGFP	**CF3640**
pRNAT-CMV3.2/Neo	CMV	Neomycin	cGFP	**CF3650**
pRNAT-CMV3.2/Hygro	CMV	Hygromycin	cGFP	**CF3660**
pRNAT-CMV3.2/Puro	CMV	Puromycin	cGFP	**CF3680**

miRNA construction service

We can assist you with the construction of the gene sequence that you have chosen and provided us : the gene can be then cloned into any of our miRNA cloning vectors, or in the vector of your choice. The resulting miRNA expressing plasmid is purified and ready to transfect and can be used for a variety of applications such as quantitation using Northern blotting, dot blotting, RNAse protection assay, primer extension analysis, Invader assay and quantitative PCR. The plasmid is sequenced in the region and sent to you along with the QC documents.

Name	Cat#	
MiRNA construction service in the vector of your choice	**CF3980**	SC1080
MiRNA construction service	**CF3980**	SC1080

Technical tip – MicroRNAs

MicroRNAs or miRNAs are endogenous, single-stranded small RNAs ~ 22 nt in length that are believed to play a crucial role in eukaryotic development by controlling post-transcriptional gene expression. They are encoded in plant and animal genomes, and are synthesized as precursors of longer hairpin molecules known as pri-miRNAs that are then processed in the nucleus to release 60-70 nt pre-miRNA hairpins.

miRNAs, due to their low abundance, are difficult to isolate and once isolated, these 18-24nt RNAs have to be purified, ligated to 5' and 3' adapter sequences and after RT-PCR cloned into vectors and sequenced. In place of this, computational analysis from genomic sequences is often used as a valuable tool that complements cloning. New miRNAs are identified in this manner that should correlate with computational analysis.

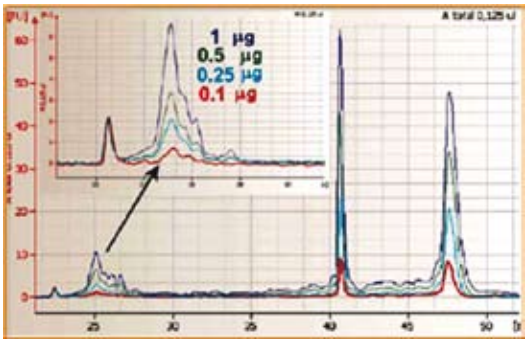
vector based microRNA technology (miRNA)

miRNA analysis with Agilent Bioanalyser

Small nucleic acids ranging in size from 6 to 150 nucleotides can be analyzed running the Small RNA assay on the Agilent 2100 bioanalyzer, for verifying sample integrity. The Small RNA assay can :

- Quantify miRNA in the concentration range of 50 - 2000 pg/µL relative to an external standard, for verifying sample enrichment and purity
- Determine the integrity and the concentration of ARN 5.8Sn 5S and tRNA, but also the microRNAs in primitive form (pri-miRNA), precursor (pre-mi-RNA) and mature (miRNA)
- Automatize sample quantitation, sizing and purity determination

Please ask for the application note, and contact us for more information about Agilent BioAnalyser system and RNA detection kits.



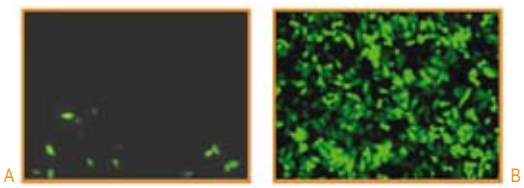
Electrophoregram fo RNA samples from 1µg to 0.125µg. Insert is for small and microRNA (<200pb).

Name	Cat#	Qty
Agilent 2100 Small RNA Kit (Chip Kit) Includes reagents, 25 chips, 1 cleaning chip, 1 syringe and reagent kit guide. The Small RNA Kit includes reagents, ladder and markers. It is not required to order these items separately.	**5067-1548**	25 chips

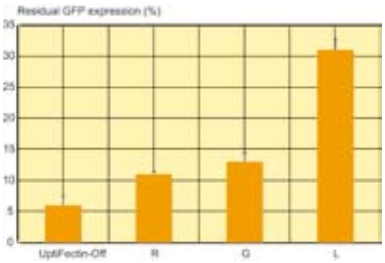
UptiFectin™ Transfection agent for Protein silencing (siRNA)

- Superior efficiency siRNA delivery in a variety of cells
- Works also in presence of serum
- Proven absence of toxicity at the effective concentrations
- Removal of transfection complex is not needed
- Particularly suitable for primary and stem cell transfection
- Results in silencing of greater than 95% of target gene expression
- One step, serum compatible reagent

UptiFectin-Off is a new synthetic derivative of a natural compound forming liposomes designed specifically for high efficiency siRNA delivery in a variety of cells. Adherent cells are equally transfected either with forward or reverse transfection procedures. Detailed protocols are given for reverse and forward transfections, adherent cells and suspensions, as well guidelines for optimisations (scaling up/down, cell density,...). Transfection efficiency was proven in several cell lines compared with competiting products (HeLa, KEK, H1299, Hep3B,...). As a result UptiFectinOFF is an ideal and cost-effective reagent for research to screening applications.



Fluorescence microscopy visualization of GFP silencing. The GFP-expressing human lung cancer H1299 cells were transfected with control siRNA (left) or anti-GFP siRNA (right) (37.5 ng/well) formulated using UptiFectin-OFF reagent. After 24 hours, the transfected H1299 cells were observed using a FITC filter to visualize GFP fluorescence.



The GFP targetting siRNA (37.5ng/well) was transfected using UptiFectin™ OFF or competitor agents (X, H, L). The GFP expression is clearly inhibited at lower levels using UptiFectinOFF. This efficiency was also shown by measuring the GFP mRNA levels by RT-PCR (not shown), that were lowered down 4.1%.

Name	Cat#	Qty
UptiFectinOFF Transfection Reagent provides sufficient reagent to perform up to 250 transfections in 24-well plates.	**CK5090**	0.5 ml*

Related products:
UptiFectinON #CK5060 DNA transfection reagent