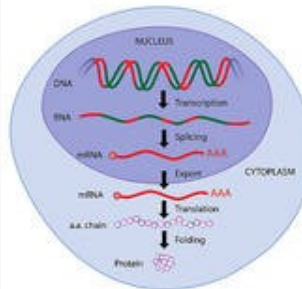
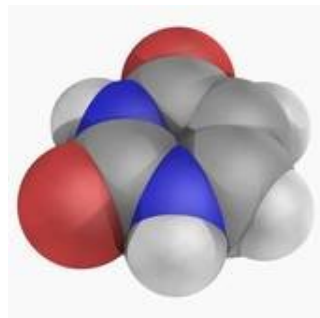


# Custom RNAi Services

## GeneCust Europe

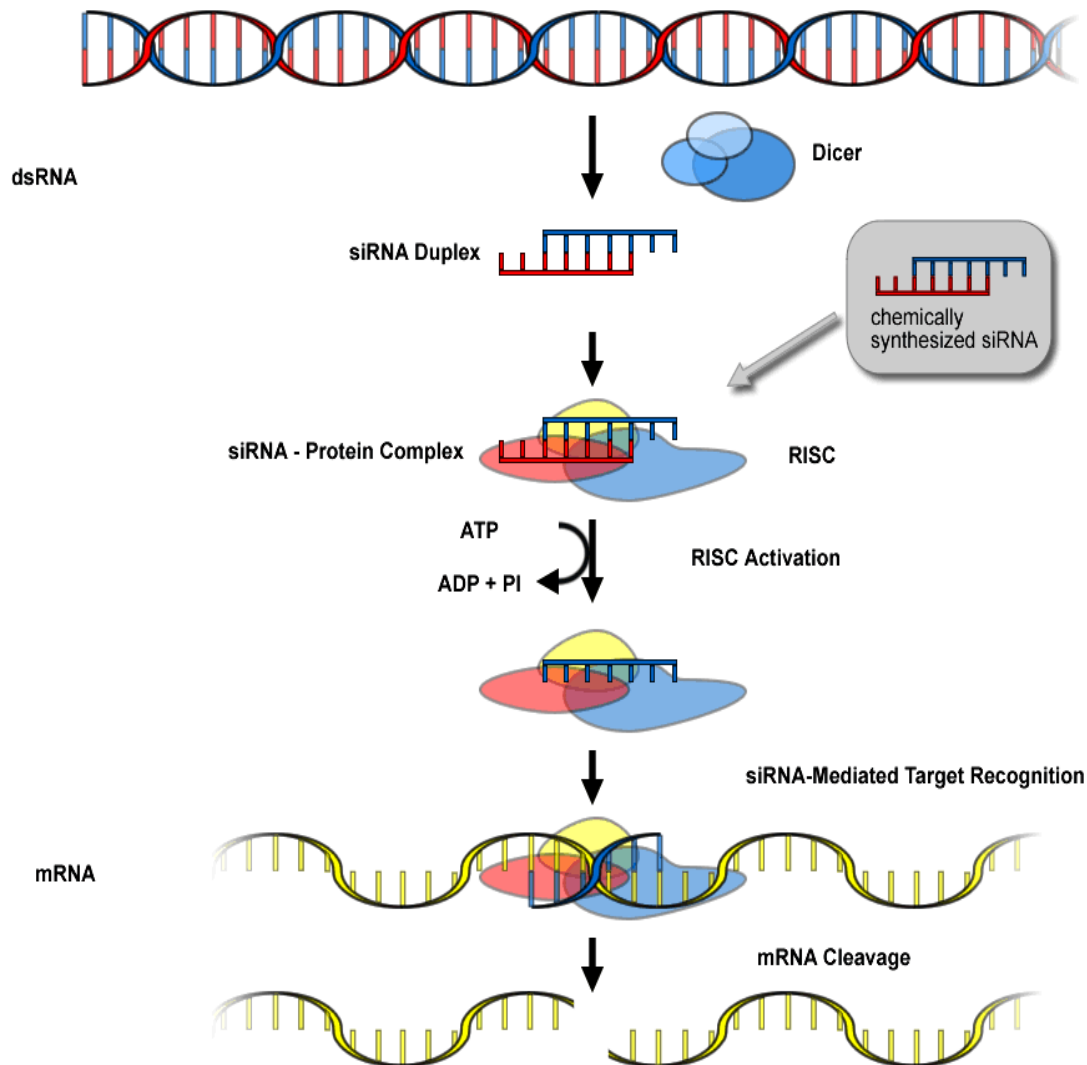


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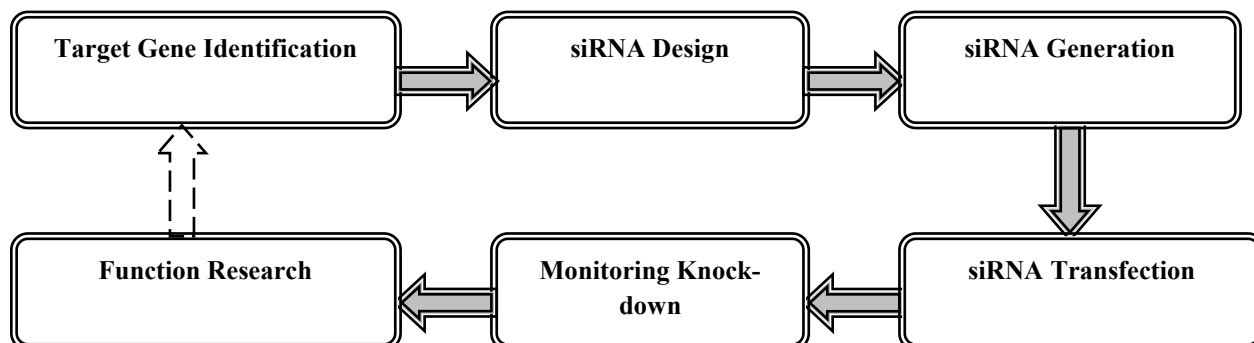
# RNAi Introduction

## RNAi Experiment Machinery

RNA (RNA interfering, RNAi) phenomenon was first observed when RNA was shown to inhibit protein expression in plants and fungi as post-transcriptional gene silencing and quelling. In 1998, Fire and Mello first observed that double-stranded RNA was the source of sequence-specific protein inhibition in *C.elegans* known as RNA interference. While the studies in *C.elegans* were encouraging, RNAi was limited in use to lower organisms because delivering long dsRNA for RNAi was non-specifically inhibitory in mammalian cells. Further studies in plants and invertebrate animals demonstrated that actual molecules that lead to RNAi were short double-stranded RNA oligonucleotides, 21 to 22 nucleotides in length, processed internally by an enzyme called Dicer. The Dicer products are referred to as short (or small) interfering RNA and are today popularly known as siRNA



## RNAi Workflow Solution



## GeneCust siRNA characteristics

GeneCust silencing product line covers the chemical synthesis of RNA monomers, common siRNA oligos and chemically modified RNA oligos; shRNA; DNA coding shRNAs, plasmid vector coding shRNAs. GeneCust Europe uses high-throughput synthesizers and state-of-the-art HPLC purification equipment.

### Quality control :

All our oligos undergo vigorous process monitoring and strict quality control; They are produced according to our standard operating procedures; All products are strictly quantified by spectrophotometry.

### Purification :

HPLC; siRNA content >97%

### Modification :

3' and 5' end Biotin, FAM and Phosphate

### Length :

19~23 base/each dsRNA

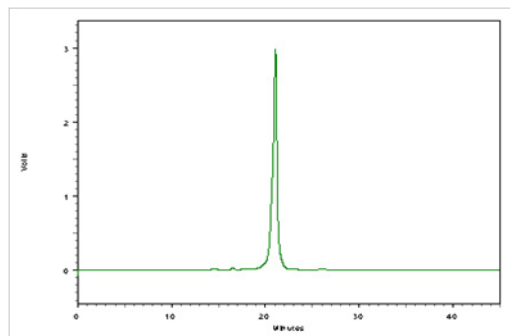
### Storage and stability :

Although oligonucleotides are stable in solution at 4°C for up to 2 weeks, GeneCust recommends storage at -20°C. Repetitive freeze-thaw cycles should be avoided by storing as aliquots. Storing at concentrations above 20 µM is recommended. GeneCust guarantees its oligonucleotides for 6 months, stored under above conditions.

### Technical data sheet :

Oligonucleotides are delivered with an Oligonucleotide Technical Data Sheet, that includes oligo name, sequence, concentration, quantity in OD and nmols, T<sub>m</sub>, MW, size, extinction coefficient and purification data.

### Free design support



## Custom siRNA oligos Price List

Cat#	Product	Quantity	Price Euros
A01001	Custom siRNAs	2 OD	73
A01002	Custom siRNAs	3 OD	87
A01003	Custom siRNAs	4 OD	103
A01004	Custom siRNAs	5 OD	123
A01005	Custom siRNAs	6 OD	144
A01006	Custom siRNAs	7 OD	165
A01007	Custom siRNAs	8 OD	186
A01008	Custom siRNAs	9 OD	206
A01009	Custom siRNAs	10 OD	227
A01010	Custom siRNAs	25 OD	515
A01011	Custom siRNAs	50 OD	990
A01012	Custom siRNAs	100 OD	1650
A01013	Custom siRNAs	250 OD	3093
A01014	Custom siRNAs	500 OD	Inquire
A01015	Custom siRNAs	1250 OD	Inquire
A01016	Custom siRNAs	2500 OD	Inquire
A02001	Chemically-Modified siRNAs (2'-O-Me or 2'-Fluoro)	2 OD	93
A02002	Chemically-Modified siRNAs (2'-O-Me or 3'-Fluoro)	3 OD	109
A02003	Chemically-Modified siRNAs (2'-O-Me or 4'-Fluoro)	4 OD	124
A02004	Chemically-Modified siRNAs (2'-O-Me or 5'-Fluoro)	5 OD	144
A02005	Chemically-Modified siRNAs (2'-O-Me or 6'-Fluoro)	6 OD	165
A02006	Chemically-Modified siRNAs (2'-O-Me or 7'-Fluoro)	7 OD	196
A02007	Chemically-Modified siRNAs (2'-O-Me or 8'-Fluoro)	8 OD	217
A02008	Chemically-Modified siRNAs (2'-O-Me or 9'-Fluoro)	9 OD	248
A02009	Chemically-Modified siRNAs (2'-O-Me or 10'-Fluoro)	10 OD	268
A02010	Chemically-Modified siRNAs (2'-O-Me or 11'-Fluoro)	25 OD	556
A02011	Chemically-Modified siRNAs (2'-O-Me or 12'-Fluoro)	50 OD	1031
A02012	Chemically-Modified siRNAs (2'-O-Me or 13'-Fluoro)	100 OD	1752
A02013	Chemically-Modified siRNAs (2'-O-Me or 14'-Fluoro)	250 OD	3299
A02014	Chemically-Modified siRNAs (2'-O-Me or 15'-Fluoro)	500 OD	Inquire
A02015	Chemically-Modified siRNAs (2'-O-Me or 16'-Fluoro)	1250 OD	Inquire
A02016	Chemically-Modified siRNAs (2'-O-Me or 17'-Fluoro)	2500 OD	Inquire
A03001	Fluorescently-labeled siRNAs (FAM or Cy3)	2 OD	135
A03002	Fluorescently-labeled siRNAs (FAM or Cy3)	3 OD	151
A03003	Fluorescently-labeled siRNAs (FAM or Cy3)	4 OD	165
A03004	Fluorescently-labeled siRNAs (FAM or Cy3)	5 OD	185
A03005	Fluorescently-labeled siRNAs (FAM or Cy3)	6 OD	206
A03006	Fluorescently-labeled siRNAs (FAM or Cy3)	7 OD	248
A03007	Fluorescently-labeled siRNAs (FAM or Cy3)	8 OD	268

Cat#	Product	Quantity	Price Euros
A03008	Fluorescently-labeled siRNAs (FAM or Cy3)	9 OD	309
A03009	Fluorescently-labeled siRNAs (FAM or Cy3)	10 OD	330
A03010	Fluorescently-labeled siRNAs (FAM or Cy3)	25 OD	660
A03011	Fluorescently-labeled siRNAs (FAM or Cy3)	50 OD	1195
A03012	Fluorescently-labeled siRNAs (FAM or Cy3)	100 OD	1855
A03013	Fluorescently-labeled siRNAs (FAM or Cy3)	250 OD	3712
A05001	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	2 OD	155
A05002	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	3 OD	171
A05003	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	4 OD	186
A05004	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	5 OD	206
A05005	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	6 OD	227
A05006	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	7 OD	278
A05007	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	8 OD	299
A05008	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	9 OD	351
A05009	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	10 OD	371
A05010	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	25 OD	701
A05011	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	50 OD	1238
A05012	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	100 OD	1959
A05013	Chemically-Modified (2'-O-Me or 2'-Fluoro) & Fluorescently-labeled siRNAs	250 OD	3919
A06001	negative control siRNA	1 OD	24
A07001	FAM-labeled Negative Control siRNA	1 OD	31
A08001	Positive Control siRNA, LaminA/C	1 OD	24
A08002	Positive Control siRNA, GFP274	1 OD	24
A08003	Positive Control siRNA, Luciferase GL2	1 OD	24
A08004	Positive Control siRNA, MAPK1	1 OD	24
A08005	Positive Control siRNA, Beta-Actin	1 OD	24
A08006	Positive Control siRNA, Vimentin	1 OD	24
A08007	Positive Control siRNA, P53	1 OD	24
A08008	Positive Control siRNA, GAPDH	1 OD	24
A08009	Positive Control siRNA, Cyclophilin B	1 OD	24

## RNAi SET

We recently launched our RNAi set service. You only have to provide the gene (target) sequence and we help you to design 4 siRNA pairs. We guarantee that at least one pair will effectively suppress gene expression .

The RNAi Sets Includes :

1. 4 pairs of corresponding gene siRNA, HPLC purification, 10nmol
2. Negative control, 1 pair, HPLC purification, 1 OD
3. Negative control, FAM labelled siRNA, 1 pair, HPLC purification, 1 OD
4. Positive control GAPDH, 1 pair, HPLC purification, 1 OD

This package also includes a free synthesis of four additional duplexes if no obvious knockdown result (60%) is achieved with the first four duplexes. We will just charge our standard shipping fee.

Cat#	Product	Quantity	Price Euros
SI-SET	RNAi Set	1 package	450

## Vector Based RNAi Services

siRNA expression vectors with antibiotic marker may suppress target gene expression in the long term, lasting for several weeks, or even longer.

If you need to maintain gene silencing for a long time, we recommend to use a shRNA vector system. GeneCust is devoted to provide the most advanced and the most convenient shRNA correlated tools. We promote new generation of shRNA expression plasmids, in highly effective ready-to-use vectors. The vector is able to stably produce shRNAs in cell, then allows for long term silencing.

### shRNA expression vectors system characteristics

1. The cloning vector has two cloning enzymes cleavage site : BamH I and BbsI. BbsI is a special restriction enzyme, it produces asymmetrical complementary coherent terminals that guarantee directional insertion and prevents vector from self-circularization.
2. shRNA vector has selection marker, which may help establish stable transfection cell line  
Neo: Neomycin resistant gene  
Hygro: Hygromycin B resistant gene  
GFP-Neo: GFP reporter and Neomycin resistant gene
3. Reporter GFP may help to detect transfection efficiency and to localize RNAi action sites.

### shRNA vectors

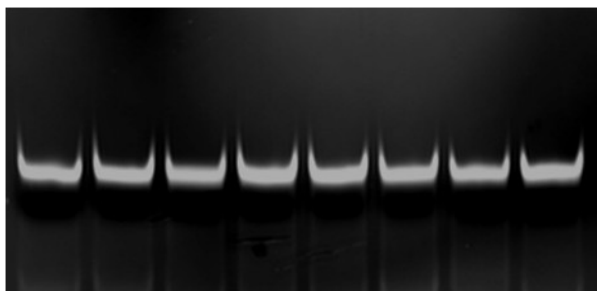
A vector set includes shRNA expression vectors, as well as corresponding negative control vector, and GFP positive control vector.

Ref.	Product	Promotor	Selection	Quantity	Price
E-01	pGPU6	U6	—	50 µg	€415
E-02	pGPH1	H1	—	50 µg	€415
E-03	pGPU6/Neo	U6	Neo	50 µg	€415
E-04	pGPH1/Neo	H1	Neo	50 µg	€415
E-05	pGPU6/Hygro	U6	Hygro	50 µg	€415
E-06	pGPH1/Hygro	H1	Hygro	50 µg	€415
E-07	pGPU6/GFP/Neo	U6	Neo	50 µg	€580
E-08	pGPH1/GFP/Neo	H1	Neo	50 µg	€580

## Custom shRNA expression vector

We may help you to construct your plasmid by inserting a validated shRNA. We can also offer accurate sequence control. You only have to tell us the target gene sequence or Gene ID number and name of the insertion vector, and we will send you the construct. We will provide shRNA expression vector in high purity quality.

Ref	Product	Promotor	Selection	Quatification	Price	Delivery time
F-01	pGPU6	U6	—	50 µg	€385	3 weeks
F-02	pGPH1	H1	—	50 µg	€385	3 weeks
F-03	pGPU6/Neo	U6	Neo	50 µg	€500	3 weeks
F-04	pGPH1/Neo	H1	Neo	50 µg	€500	3 weeks
F-05	pGPU6/Hygro	U6	Hygro	50 µg	€500	3 weeks
F-06	pGPH1/Hygro	H1	Hygro	50 µg	€500	3 weeks
F-07	pGPU6/GFP/Neo	U6	Neo	50 µg	€670	3 weeks
F-08	pGPH1/GFP/Neo	H1	Neo	50 µg	€670	3 weeks



**What is the best location on the duplex to incorporate a dye label?**

Modifications on the 5'-end of the antisense strand have shown to interfere with siRNA silencing activity and therefore this position is not recommended for modification. Modifications at the other three termini have been shown to have minimal to no effect on silencing activity. Modification on the 5'-end of the sense strand is the position that provides the most efficient chemical synthesis and thus is recommended.

**If I have the protein sequence for a particular gene product, can an siRNA be designed to target this protein?**

siRNAs function at the mRNA level, not at the protein level. In order to design siRNA, the precise target mRNA nucleotide sequence is required. Due to the degenerate nature of the genetic code and codon bias, it is impossible to predict accurately the correct nucleotide sequence from the peptide sequence. Additionally, since the function of siRNAs is to cleave mRNA sequences, it is important to use the mRNA nucleotide sequence and not the genomic sequence for siRNA design. Pre-mRNA processing may remove intron sequences which are also found in the genomic sequence. Designs using genomic information may inadvertently target introns and as a result the siRNA may not be functional for silencing the corresponding mRNA.

**Will an siRNA designed to target the human version of a particular gene work in a different species?**

siRNAs designed specifically to target human genes are not expected to silence homologs in other species. Cross-targeting in other species is observed rarely, even when a high identity existed between the gene sequences. But under certain conditions siRNAs can be specifically designed to be functional in two or more species. This involves careful siRNA design and bioinformatics analysis. Please contact us for more information.

**What is the best method of delivering siRNAs into the cell?**

There are several methods that are used for delivering siRNAs into cells including lipid-based transfection, electroporation, calcium phosphate co-precipitation, microinjection and vector delivery techniques. The choice between these methods is often a result of several factors including the ability of the cells to tolerate the delivery method, susceptibility to viral infection, and the growth properties of the cells. Although lipid-based transfection is one of the more commonly used methods for adherent cells, suspension cells are often more difficult to transfect and generally have higher rates of delivery with electroporation techniques.

**Which transfection reagent is recommended for delivery of siRNA?**

The choice of a transfection reagent often depends more upon the particular cell line than the substance being delivered into the cells. We recommend to use a reagent that has been previously used in the cells of interest. Further optimization for siRNA delivery may be necessary. If no established protocol for the cells is available, a PubMed or HighWire search may help identify if a published protocol for the specific cell line or a similar cell line has been reported. In all instances, we recommend following the protocol provided by the manufacturer of the transfection reagent.

**Is there a quick method for monitoring transfection efficiency?**

GeneCust has developed several options including a family of fluorescent-labeled siRNAs and a novel RNA-based reagent known as siRNA Transfection Control. The uptake of siRNAs can be visualized with the appropriate filters on a confocal microscope or by flow cytometry. Alternatively, fluorescent-labeled siRNA is cytotoxic when successfully delivered into cells. Cells that have efficiently taken up this transfection control typically undergo apoptosis within 24 to 48 hours. This phenotypic outcome can easily be monitored using standard cell viability methods (e.g., alamarBlue, MTT cytotoxicity, Trypan Blue dye exclusion, JC1 dye, or other appropriate assays).

**I see a fair amount of cell death when I transfect. What can I do about this?**

Extensive cell death following transfection is an indication that delivery conditions need to be further optimized. Basic parameters to consider when optimizing transfections include transfection reagent and cell specific conditions such as the ratio of siRNA:lipid reagent, the lot/batch of transfection reagent, duration of transfection, cell passage number and cell density at transfection. Often decreasing the amount of lipid present during transfection and/or the total duration of transfection will help minimize the toxic effect to the cells. Additionally, it is not uncommon to observe significant variability from one tube of transfection reagent to another, and this may also represent a source of experimental variability. If the problem persists, we recommend that other transfection reagents be considered.



**Why is optimizing transfection efficiency important?**

Transfection efficiency is a measure of the proportion of cells that successfully internalized the siRNA. Low transfection efficiency will result in lower observed silencing, not because the siRNA is a poor silencer, but because mRNA expression from untransfected cells will contribute to the total observed mRNA level.

**I transfected my siRNA and I'm seeing less than 75% silencing at the mRNA level. What is wrong?**

The two most frequent causes of poor silencing are low transfection efficiency and poor siRNA sequence design. Often the lack of silencing is related to inefficient delivery of siRNA into the cells, suggesting that transfection conditions need to be optimized. If these procedures to optimize transfection conditions have already been performed and poor or moderate silencing persists, an alternative transfection reagent or technique (e.g., electroporation) may provide better delivery for that particular cell line.

If transfection efficiencies have been optimized and poor silencing results are still observed, the siRNA sequence design may be the cause. Conventional siRNA design rules and publicly available design tools vary significantly in their ability to select functional sequences for difficult-to-silence genes. In addition, poor to moderate siRNA duplexes have been shown to exhibit variability in silencing efficiency.

**What is the best method for monitoring siRNA functionality?**

siRNA-mediated silencing occurs as a result of target mRNA recognition and cleavage. The status of the target mRNA level is critical for understanding the experimental system and for isolating potential issues while troubleshooting. Because of the variability in protein stability and turnover rates in biological systems, the time course and degree of protein reduction may differ significantly from that of the target mRNA. Thus, while protein measurement provides important experimental information, measurement of the silencing effect at the mRNA level is the most important and reliable indication of siRNA-mediated silencing efficiency. Once successful silencing has been confirmed at the mRNA level, subsequent measurement of target protein levels can assist in correlating mRNA reduction with phenotypic effects. Time-course studies to assess maximal protein reduction may need to be performed to further optimize transfection protocols for protein-level studies.

**I plan on using RT-PCR to detect knockdown of my target gene expression. Is there anything I need to take into consideration when designing my primers?**

We recommend that primers be designed to bracket one of the siRNA cleavage sites as this will help eliminate possible bias in the data (i.e., one of the primers should be upstream of the cleavage site, the other should be downstream of the cleavage site). Bias may be introduced into the experiment if the PCR amplifies either 5' or 3' of a cleavage site, in part because it is difficult to anticipate how long the cleaved mRNA product may persist prior to being degraded. If the amplified region contains the cleavage site, then no amplification can occur if the siRNA has performed its function.

**What is the best time-frame for monitoring siRNA-dependent decreases in target mRNA expression levels? In target protein levels?**

Generally, target mRNA levels are decreased after 24 hours post-transfection following transfection of a gene-specific siRNA duplex. However, maximal silencing may be reached at a later time point, so it is advisable to assay target mRNA in a time-course study. In most cases, silencing will be maximal at 24 to 48 hours following transfection.

Cellular target protein levels should be examined starting at 24 hours and assayed until a minimum level is noted, often 48 to 96 hours or greater. As always, it is important to verify transfection efficiency using an appropriate positive control. If there is no decrease in protein levels within this time frame, it may be necessary to perform a second siRNA transfection, use a stabilized siRNA, or develop a vector-based silencing cassette that can continuously produce the siRNA for extended periods of time.

**I transfected my siRNA at 100 nM and only saw 50% silencing. Should I increase the siRNA concentration to 200 nM or even 400 nM?**

Increasing the concentration of a moderately functional siRNA generally does not improve silencing ability. In addition, higher concentrations of siRNA can lead to significant off-target effects and can ultimately be toxic to the cells. In contrast, highly functional siRNAs that result from rational design algorithms typically generate 75% or greater silencing at 100 nM or lower concentrations. We recommend that the concentration and integrity of the siRNA be assessed as a first step in troubleshooting. The absorbance at 260 nm should be used together with the provided extinction coefficient to confirm concentration. The integrity of the duplex may be verified on a non-denaturing PAGE where a single band representing the duplex should be observed. Finally, the lack of significant silencing can be a result of poor transfection efficiency, suggesting that siRNA delivery should be further optimized.

**How are fluorescently labeled siRNAs detected?**

Fluorophore labeled duplexes are popular items for optimizing transfection conditions. Uptake of the labeled pre-synthesized siRNAs is readily detected either by flow cytometry or by fluorescence confocal microscopy. We recommend that you use protocols and procedures that are specific for your optical system and software package to detect the fluorescently labeled cells. It is also important to consider that fluorescence may not correlate with siRNA function unless the siRNA has been modified specifically for nuclease resistance.

**How do I calculate the amount of µg siRNA in each well if I use 6 µL of a 10 µM stock siRNA solution?**

There are several steps to completing this calculation. First, calculate how many nmol siRNA are in each well:

- equation:  $? \text{ nmol} = (6 \text{ } \mu\text{L})(10 \text{ } \mu\text{mol/L})$
  - unit conversions:  $? \text{ nmol} = (6 \text{ } \mu\text{L})(10 \text{ } \mu\text{mol/L})(1 \text{ L}/10^6 \text{ } \mu\text{L})(1000 \text{ nmol}/\mu\text{mol})$
  - answer:  $? \text{ nmol} = 0.06 \text{ nmol}$
- Therefore, there is 0.06 nmol of siRNA in each well.

Next, use the molecular weight of siRNA to convert between nmol and µg. If the specific molecular weight is not known, you may use the average molecular weight of siRNA, which is 13,300 g/mol.

- equation:  $? \text{ } \mu\text{g} = (0.06 \text{ nmol})(13,300 \text{ g/mol})$
  - unit conversions:  $? \text{ } \mu\text{g} = (0.06 \text{ nmol})(13,300 \text{ g/mol})(\text{mol}/10^9 \text{ nmol})(10^6 \text{ } \mu\text{g/g})$
  - answer:  $? \text{ } \mu\text{g} = 0.798 \text{ } \mu\text{g}$ , or  $0.8 \text{ } \mu\text{g}$
- Therefore, there is 0.8 µg of siRNA in each well, when 6 µL of a 10 µM siRNA stock solution is used.

**How do I convert between nmol and µg of siRNA?**

Use the molecular weight of siRNA to convert between nmol and µg. If the specific molecular weight is not known, you may use the average molecular weight of siRNA, which is 13,300 g/mol.

If, for example, you have 5 nmol of siRNA, the conversion would be performed using the following steps:

- equation:  $? \text{ } \mu\text{g} = (5 \text{ nmol})(13,300 \text{ g/mol})$
  - unit conversions:  $? \text{ } \mu\text{g} = (5 \text{ nmol})(13,300 \text{ g/mol})(\text{mol}/10^9 \text{ nmol})(10^6 \text{ } \mu\text{g/g})$
  - answer:  $? \text{ } \mu\text{g} = 66.5 \text{ } \mu\text{g}$
- Therefore, a 5 nmol quantity of siRNA is 66.5 µg.

**About positive and negative controls**

A positive control is useful as an experimental systems check. That is, when you see the expected results with a positive control siRNA, you have reasonable assurance that your transfections, as well as your RNA extraction and assay, are reliable and robust in your experimental model.

A non-targeting siRNA control is important to help establish that any decrease in gene expression levels observed with a gene-specific siRNA is related to a sequence-specific RNAi event. Global down-regulation events may be due to cellular stress responses to a certain transfection reagent or technique. Without negative controls, a researcher might mistakenly interpret this broad, non-specific silencing as true gene-specific silencing. To control for non-RNAi related or non-specific effects, GeneCust offers a variety of products for RNAi experiments.

**I heard I should use a scrambled control that has the same GC content as my siRNA. Isn't that better than a random sequence?**

A true scrambled control containing the same base composition as your target specific siRNA might be considered a good non-specific control, provided that the scrambled siRNA has been verified to not inadvertently target another gene. However, experimental data indicate that even when no known target is identified, certain non-targeting siRNAs result in some cellular toxicity. With respect to preserving the overall base content (or GC content), GeneCust has found that this is less critical as the relative position of the bases will also have an impact on interactions with the silencing machinery. Until a better method is available for predicting the potential for toxicity or non-specific effects, it is recommended that a non-targeting siRNA that has been validated and determined to be non-functional or "inert" be used as a negative control.

**How do I store my siRNA samples?**

siRNAs should be stored at -20°C or -70°C in a non-frost free freezer, either as a dried pellet or resuspended in an RNase-free solution buffered to pH 7.4 - 7.6 to help with stability during freeze-thaw cycles. We recommend that the siRNAs be resuspended to a convenient stock concentration (20-100 µM) and stored in small aliquots to avoid multiple freeze thaw cycles. siRNAs should not go through more than 5 freeze thaw cycles. When stored under these conditions and using good RNase-free technique, they typically remain stable for more than 6 months. If degradation is a concern, the integrity of the siRNA duplexes can be evaluated on an analytical PAGE gel.

# GeneCust

**Custom Services for Research**



Thank you for your time.