



## Product Specifications

Guaranteed RNAi Explorer kit with Fluorescein/Dabcyl Molecular Beacon □ Catalog No.:27-6402-01  
Guaranteed RNAi Explorer kit with Fluorescein/Tamra TaqMan □ Catalog No.:27-6402-01  
Guaranteed RNAi Explorer kit with 6 oligos up to 30mer 50 nmol scale □ Catalog No.:27-6402-01  
Custom siRNA synthesis □ Catalog No.:27-6401-0X

# RNAi Explorer™

## RNA Interference

### RNAi Explorer™

RNAi Explorer™ from Gene Link is a series of product and services to aid researchers in exploring RNA interference. The online search and design algorithms for siRNA or shRNA are developed based on current known guidelines. You can place an order for the Guaranteed RNAi Explorer™ kit by simply providing Gene Link the appropriate Gene ID or Accession Number of your gene of interest. Alternatively, you can design your own siRNA or shRNA at Gene Link's website.

#### siRNA Synthesis

The siRNA duplexes provided are synthesized as a single strand oligo and annealed after complete deprotection and purification steps are completed. Gene Link utilizes the most reliable standard RNA synthesis chemistry. The RNA duplex is supplied lyophilized and ready to use.

#### Handling & Storage

Follow established stringent RNase free handling conditions. The lyophilized siRNA duplex should be stored immediately at -20° C. The lyophilized siRNA is stable for ~6 months at -20° C.

### □ Guaranteed RNAi Explorer™ Kit

Please consult and save enclosed 'Order Specifications' sheet, this gives your gene specific siRNA information. This kit contains the following.

1. 3 siRNA duplexes 10 nmols each of 19+2 dT 3' overhangs. Supplied lyophilized, ready to dissolve and use. Please see order report for sequence information.
2. 1 negative control non-silencing siRNA duplex. 5 nmols supplied lyophilized, ready to dissolve and use.
3. Choice of detection probe or custom oligos if requested at time of order.

Dissolve the lyophilized siRNA duplex in RNase-free deionized water as follows to give a final concentration of 20 μmolar.

siRNA duplex	RNase-free deionized water to add
Gene Specific siRNA	500 μl
Negative Control siRNA	250 μl

Incubate the siRNA at 90° C for 1 minute, then at 37° C for 60 minutes.

*Guarantee: Gene Link guarantees a minimum of 70% silencing of your gene with at least one of the siRNA supplied. Gene Link will synthesize another set of 3 siRNA if evidence is provided that none of the provided siRNA was functional in achieving a minimum of 70% silencing.*

### □ Custom siRNA Synthesis

Please consult and save enclosed 'Order Specifications' sheet, this gives order specific data including the exact nmol quantity supplied of each duplex.



## Preparation of siRNA for Transfection

Dissolve the lyophilized siRNA duplex in RNase-free deionized water as follows to give a final concentration of 20  $\mu$ molar. Incubate the siRNA at 90° C for 1 minute, then at 37° C for 60 minutes. Once it is dissolved, the siRNA should be aliquoted and stored at -80° C. For long term storage the siRNA can be ethanol precipitated in the following manner:

1. Add 1/10<sup>th</sup> volume of 10 M ammonium acetate.
2. Add 3 volumes of absolute ethanol and vortex briefly.
3. Store at -80° C.
4. When ready for use centrifuge at 14,000 x g for ten minutes.
5. Remove supernatant and leave the tube open to the air for 30 minutes in order to allow any residual alcohol to evaporate.
6. Dissolve in RNase-free deionized water as previously described.

## Cell Transfection

1. Twenty-four hours prior to transfection, incubate a 24-well tissue culture plate with 2-10 x 10<sup>4</sup> exponentially growing adherent cells per well.
2. Grow the cells overnight in an appropriate volume of growth medium. Cells should be approximately 40-70% confluent at the time of transfection.
3. Combine the siRNA and transfection reagent\* according to the manufacturers specifications. In most cases this should be done in the absence of serum and antibiotics.
4. Remove the old medium from the cells and replace with fresh serum-containing medium.
5. Add the siRNA-transfection reagent complex.
6. Incubate the cells from 4-72 hours.
7. Perform appropriate assays to assess gene expression.

Partial list of commercially available transfection reagents: siPORT Amine Transfection Reagent from Ambion; BLOCK-IT Dicer RNAi Transfection kit from Invitrogen; TransIT-TKO siRNA Transfection Reagent from Mirus; RNAifect Transfection Reagent from Qiagen; GeneEraser siRNA Transfection Reagent from Stratagene and RiboJuice siRNA transfection Reagent from Novagen.

## Controls for RNAi experiments

A negative control should also be included when performing siRNA experiments to eliminate the possibility of nonspecific silencing effects. For this purpose, Gene Link provides a siRNA sequence that has been shown to have no effect on gene silencing. The negative control siRNA is also available labeled with fluorescein, which allows transfection efficiency to be tracked by fluorescent microscopy. When monitoring transfection efficiency it should be noted that the pH indicator, phenol red, used in most culture media should be omitted as it interferes with fluorescent analysis. Transfection conditions can be optimized further by the use of positive controls which consist of siRNA sequences that have been proven to be gene specific silencers.

## Analyzing gene silencing

Gene silencing can be analyzed at either the protein or the mRNA level. Protein expression analysis is by western blotting, immunofluorescence, or FACS®. At the mRNA level gene silencing can be monitored by real-time RT-PCR, northern blotting, or microarray analysis. All gene expression data should be compared with levels of a non-related gene, such as beta actin or GAPDH, to normalize for variable amounts of RNA in different samples. Primers as well as Taqman probes and Molecular Beacons for beta actin and GAPDH are available from Gene Link.

## Primer design for RT-PCR

Primers should be designed to amplify a region of the cDNA that spans an exon-exon boundary in order to prevent amplification from contaminating genomic DNA. Furthermore, primers should be designed to amplify the region flanking the siRNA binding site as partially degraded target mRNA, while no longer functional, may act as a template for PCR.

## Troubleshooting & FAQ's

### Low transfection efficiency

- ◆ When monitoring transfection efficiency by fluorescence uptake, measurements should be performed within 4-8 hours following transfection. After 8 hours the fluorescent signal may weaken even though the siRNA remains stable.
- ◆ Confirm that the medium used for formation of the siRNA-transfection reagent complex is serum-free.
- ◆ The transfection medium also should not contain polyanions such as heparin or dextran sulfate.
- ◆ Be sure the cell density at the time of transfection is optimal. The most favorable cell density for transfection is typically 40-70% confluence.



Gene Link™

140 Old Saw Mill River Road Hawthorne, NY 10532  
Tel: 914.769.1192 www.genelink.com Fax: 914.769.1193





## RNAi Explorer™ Product Line

Guaranteed RNAi Explorer™ Kit*		
Components	Qty	Price
3 Target siRNAs	10 nmol each	\$995.00
1 negative control siRNA	5 nmol	
1 QPCR probe or 6 free oligos*	5 nmol	

\*Select choice when placing order

27-6402-01	Guaranteed RNAi Explorer kit with Fluorescein/Dabcyl Molecular Beacon
27-6402-02	Guaranteed RNAi Explorer kit with Fluorescein/Tamra TaqMan
27-6402-06	Guaranteed RNAi Explorer kit with 6 oligos up to 30mer at 50 nmol scale

siRNA (duplexed)				
Catalog Number	27-6401-06	27-6401-05	27-6401-02	27-6401-10
Purification	20 nmol scale	50 nmol scale	200 nmol scale	1 µmol scale
Crude	\$230.00	\$320.00	\$380.00	\$800.00
RPC	\$325.00	\$380.00	\$470.00	\$900.00
Gel	\$410.00	\$480.00	\$570.00	\$955.00

Control siRNA Duplexes			
Product	Size	Catalog Number	Price, \$
Negative Control unlabeled, non-silencing	5 nmols	27-6410-05	\$295.00
Negative Control FL labeled, non-silencing	2 nmols	27-6410-02FL	\$295.00
<b>Positive Controls, Unlabeled</b>			
Human Vimentin; NM_003380	5 nmols	27-6412-05	\$295.00
Human Beta Actin; NM_001101	5 nmols	27-6413-05	\$295.00
Mouse Beta Tubulin; AF312873	5 nmols	27-6414-05	\$295.00
Rat Chromogranin-A; NM_021655	5 nmols	27-6415-05	\$295.00
<b>Positive Controls, Fluorescein Labeled</b>			
Human Vimentin; NM_003380; FL labeled	2 nmols	27-6412-02FL	\$295.00
Human Beta Actin; NM_001101; FL labeled	2 nmols	27-6413-02FL	\$295.00
Mouse Beta Tubulin; AF312873; FL labeled	2 nmols	27-6414-02FL	\$295.00
Rat Chromogranin-A; NM_021655; FL labeled	2 nmols	27-6415-02FL	\$295.00

shRNA (DNA oligonucleotides)		
Product	200 nmol scale	1 µmol scale
DNA Oligo Synthesis	\$2.00/base	\$3.75/base
Gel Purification	\$75.00	\$150.00

**\*RNAi and siRNA**

RNA interference (RNAi) is a specific and sequence dependent targeted gene silencing activity. RNAi acts by post transcriptional degradation of mRNA by small interfering RNAs (siRNA's) of the same sequence. The silencing approaches 100% and has to be empirically determined and optimized. Not every siRNA can effectively down regulate a gene. The process of RNA interference varies by individual siRNA while some do not exhibit any interference at all. *Prices subject to change without notice* All Gene Link products are for research use only



140 Old Saw Mill River Road Hawthorne, NY 10532  
Tel: 914.769.1192 www.genelink.com Fax: 914.769.1193