Product Specifications

Guaranteed RNAi Explorer kit with Fl/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 ❑ Catalog No.:27-6402-01
Custom siRNA synthesis ❑ Catalog No.:27-6401-0X

siRNA Duplexes & RNAi Explorer™

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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.

Material Supplied

Custom siRNA are synthesized at different scales of synthesis, the yield varies between these synthesis scales, individual synthesis, modifications and purification methods. Your custom siRNA duplex is accompanied by a Certificate of Analysis stating the exact yield in nmols and µg. The exact specifications are also printed on the tube label. Please refer to the Certificate of Analysis or the tube label for the exact specifications.

Preparation of siRNA for Transfection

Dissolve the lyophilized siRNA duplex in RNase-free deionized water as follows to give a final concentration of 20 µmolar.
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 1/10th 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 10^4 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.
- Optimize the amount of transfection reagent by using a range of concentrations according to the manufacturer's recommendations.

Small or no gene silencing effect

- Monitoring of gene expression may be too soon to observe changes. This is particularly true if levels of protein expression are being monitored. A time course experiment should be performed to determine the optimal time for analysis.

- Perform a positive control experiment using a siRNA duplex that has been shown to be effective in gene silencing. Positive control siRNAs for human, mouse, and rat cell lines are available from Gene Link.

What are my siRNA delivery options?

Delivery of siRNA directly in cells can be achieved by using microinjection or electroporation. Another popular option is the use of transfection reagent. Specialized siRNA-delivery reagents are supplied by several companies. Please consult the transfection reagent vendor's protocol for detailed information for the exact requirements and procedure. Careful optimization of variable factors should be ensured for all initial transfection experiment. It is based on this and further optimization that reproducible gene knock out results will be obtained. Usually RNAi effect is seen within 4 hours and the maximum down regulation observed in 24-48 hrs. The effect lasts several cell generations and from 4-10 days depending on cell culture type.

How does purity of synthetic siRNA's affect RNAi?

RNAi is a sequence specific chain of events. Chemical synthesis of siRNA's is based on coupling of bases to yield a particular sequence. The yield and purity depends on the coupling efficiency. Gene Link siRNA's can be used without further purification, but Gene Link recommends purified siRNA's for use in transfection.

What concentration of siRNA is most effective?

As low as 1 nM concentration of siRNA have been shown to be effective in exhibiting RNAi. Initial experiments should be done at varying concentrations from 1-10 nM. Some reports have used as high as 25nM concentrations. High quality siRNA's should be used.

How do I quantify down regulation?

RNAi down regulates a gene function without actually interacting with the gene. The subtle action is by mRNA degradation. Thus the degree of RNA interference achieved is directly proportional to the level of mature mRNA and the translated proteins. The options are:

1. Measurements of target protein (enzyme) activity. This option is suitable if a robust assay is available or has been in prior use. The assay would vary by the nature of the protein product.
2. Measurement of target mRNA level. This is the preferred method as it directly quantifies the level of mRNA. Quantitative PCR is very effective in measuring relative amount of target sequence. This can be achieved simply by SYBR green or by the use of TaqMan or Molecular Beacons.

References for further information

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2. Yu Jenn-Yah, DeRuiter, S.L, and Turner, D.L. (2002) PNAS, 99, 6047-6052.
3. Gunther, S., Hanna, D., William B., K., Nicole, R., Marc H., S., Clare M., W., Sandra L., S. and M.A., S. (2004) Mol. Biol of the Cell , 15, 256-267.
4. Yang H., H., Soung H., J. and Seung H., Y. (2003) The Journal of Biological Chemistry, 278, 40581-40589
5. Jens, H., Sayda M., E., Kim, B., Thomas, T. and Klaus, W. (2001) Journal of Cell Science, 114, 4557-4565
6. Hannon, G.J. (2002) RNA interference. Nature, 418, 244 251
7. Fire,A., Xu,S., Montgomery, M.K., Kostas, S.A., Driver, S.E. and Mello, C.C. (1998) Nature, 391, 806 811.

RNAi Explorer™ Control siRNA Duplexes

The negative and positive control siRNA duplexes serve as validation for your experiments including monitoring transfection and silencing efficiencies. These are supplied lyophilized.

Control siRNA Duplexes		
Product	**Size**	**Catalog Number**
Negative Controls non-silencing siRNA for human, mouse and rat*		
Negative Control unlabeled, non-silencing	5 nmols	27-6411-05
Negative Control unlabeled, non-silencing	20 nmols	27-6411-20
Negative Control FL labeled, non-silencing	2 nmols	27-6411-02FL
Negative Control FL labeled, non-silencing	10 nmols	27-6411-10FL
Negative Control FL labeled, non-silencing	20 nmols	27-6411-20FL

Positive Controls, Unlabeled Verified high efficiency silencing of target gene		
Human Vimentin; NM_003380	5 nmols	27-6412-05
Human Vimentin; NM_003380	20 nmols	27-6412-20
Human Beta Actin; NM_001101	5 nmols	27-6413-05
Human Beta Actin; NM_001101	20 nmols	27-6413-20
Mouse Beta Tubulin; AF312873	5 nmols	27-6414-05
Mouse Beta Tubulin; AF312873	20 nmols	27-6414-20
Rat Chromogranin-A; NM_021655	5 nmols	27-6415-05
Rat Chromogranin-A; NM_021655	20 nmols	27-6415-20
Human GAPDH; NM_002046	5 nmols	27-6416-05
Human GAPDH; NM_002046	20 nmols	27-6416-20

Positive Controls, Fluorescein Labeled Verified high efficiency silencing of target gene		
Human Vimentin; NM_003380; FL labeled	2 nmols	27-6412-02FL
Human Vimentin; NM_003380; FL labeled	10 nmols	27-6412-10FL
Human Beta Actin; NM_001101; FL labeled	2 nmols	27-6413-02FL
Human Beta Actin; NM_001101; FL labeled	10 nmols	27-6413-10FL
Mouse Beta Tubulin; AF312873; FL labeled	2 nmols	27-6414-02FL
Mouse Beta Tubulin; AF312873; FL labeled	10 nmols	27-6414-10FL
Rat Chromogranin-A; NM_021655;FL labeled	2 nmols	27-6415-02FL
Rat Chromogranin-A; NM_021655;FL labeled	10 nmols	27-6415-10FL
Human GAPDH; NM_002046; FL labeled	2 nmols	27-6416-02FL
Human GAPDH; NM_002046; FL labeled	10 nmols	27-6416-10FL
Human/Mouse/Rat GAPDH; NM_002046; FL labeled	2 nmols	27-6417-02FL
Human/Mouse/Rat GAPDH; NM_002046; FL	10 nmols	27-6417-10FL

*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.

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