GeneCopoeia™
Expressway to Discovery

9620 Medical Center Drive, Suite 101
Rockeville, MD 20850
Tel: 301-762-0888

Fax: 301-762-3888
Web: www.genecopoeia.com
Email: sales@genecopoeia.com

Protocol ● siRNA oligos

Content	Size	Storage
Target gene siRNA	2 OD (5 nmol) × 3	-20~ -80°C Stable for 12 months
Negative control	1 OD (2.5 nmol)	
FAM labeled negative control	1 OD (2.5 nmol)	
positive control	1 OD (2.5 nmol)	
DEPC water		

Description

Conventional chemically synthesized siRNA is a double-stranded small molecule RNA of 21 to 23 nt, and the product is in the form of a lyophilized powder.

Transport and Storage

The product is shipped at room temperature in the form of lyophilized powder. After receiving the product, please store it at -20~ -80°C. The lyophilized powder can be stored stably for one year.
Centrifuge it briefly before use, prepare it into 20 μM stock solution with RNase-free H_2O, and store it in aliquots to avoid repeated freeze-thaw cycles.

Table 1. Preparation method of 20 μM stock solution

siRNA(nmol)	2.5	5	10	50
Dissolved volume(μL)	125	250	500	2500

Note: 1OD duplex=2.5 nmols=40 μg

Notice

1. siRNA is attached to the tube wall in the form of a very light dry film. Centrifuge before opening the tube, then slowly open the tube cap. When dissolving, add sufficient RNase-free H_2O, then cover the tube cap and shake to dissolve.
2. Since RNA is easily degraded, in order to avoid product degradation caused by external factors (including enzymes, extreme pH or temperature conditions, etc.), all operations should strictly follow the RNA operation rules, and the tubes and gun tips used must be enzyme-free and sterile. During the experiment, the product is best placed on ice, and after use, please carefully store it at -20~ -80°C to avoid repeated freeze-thaw cycles.
3. Prepare 20 μM stock solution, please divide it according to the experimental design and use it up within 3 months after division (the longer the time, the more serious the degradation, which may lead to poor experimental reproducibility).

Cell Experiment Methods

■ Cell experiment materials

- EndoFectin™ RNAi transfection reagent or other transfection reagents with equivalent performance, siRNA
- Protein-free cell culture medium (such as Opti-MEM I™, from Life Technologies. cat# 31985-088)
- 50% confluency of cells

■ Exploring conditions

Before beginning a transfection experiment, we recommend first optimizing your transfection conditions. If you have purchased EndoFectin™ RNAi transfection reagent, you can test the amounts of EndoFectin™ RNAi transfection reagent listed in Table 2 (RNAi transfection).

Table 2. Suggested starting conditions for siRNA transfection of adherent cells.

Culture vessel	Medium volume(μl)	Range of siRNA(pmol)	Final siRNA concentration (nM)	Dosage of 20 μM siRNA(μl)	Dilution volume of siRNA or EndoFectin RNAi	Recommended dosage of EndoFectin RNAi	Range of EndoFectin RNAi
96-well plate (one well)	100	8	80	0.4	5 μL	0.4 μL	0.2-0.8 μL
	100	4	40	0.2	5 μL	0.4 μL	0.2-0.8 μL
	100	2	20	0.1	5 μL	0.4 μL	0.2-0.8 μL
	100	1	10	0.05	5 μL	0.4 μL	0.2-0.8 μL
	100	0.5	5	0.025	5 μL	0.4 μL	0.2-0.8 μL
24-well plate (one well)	500	40	80	2	25 μL	2 μL	1-4 μL
	500	20	40	1	25 μL	2 μL	1-4 μL
	500	10	20	0.5	25 μL	2 μL	1-4 μL
	500	5	10	0.25	25 μL	2 μL	1-4 μL
	500	2.5	5	0.125	25 μL	2 μL	1-4 μL
12-well plate (one well)	1000	80	80	4	50 μL	4 μL	2-8 μL
	1000	40	40	2	50 μL	4 μL	2-8 μL
	1000	20	20	1	50 μL	4 μL	2-8 μL
	1000	10	10	0.5	50 μL	4 μL	2-8 μL
	1000	5	5	0.25	50 μL	4 μL	2-8 μL
6-well plate (one well)	2000	200	100	10	125 μL	10 μL	5-20 μL
	2000	100	50	5	125 μL	10 μL	5-20 μL
	2000	50	25	2.5	125 μL	10 μL	5-20 μL
	2000	25	12.5	1.25	125 μL	10 μL	5-20 μL
	2000	12.5	6.25	0.625	125 μL	10 μL	5-20 μL

■ siRNA Transfection Method

1. Cell Culture Plating:

The day before transfection, trypsinize and count the cells. Adjust the cell concentration and plate the cells in a cell-culture vessel. The number of cells plated in each well was recommended to be 50% confluent at the time of transfection (For some cells that are sensitive to contact inhibition, the cell plating density can be appropriately reduced to reduce the cell confluency during transfection.).

Note: If an antibiotic-containing cell culture medium was used for this step, replace with pre-warmed cell culture medium without antibiotics 0.5 hours prior to transfection.

2. **RNAi-EndoFectin™** Complex Preparation:

A. Equilibrate 20μM siRNA stock solution, **EndoFectin™ RNAi** Transfection Reagent, and protein-free culture medium (e.g., Opti-MEM I™) to room temperature. Refer to Table 2 and dilute siRNA and **EndoFectin™ RNAi** transfection reagent in protein-free medium for 5 minutes at room temperature. (The volume ratio of diluted siRNA and EndoFectin™ RNAi transfection reagent solution is 1:1)

(For example: For siRNA transfection in 1 well of 6-well plate, pipette 1.25 μL siRNA from the 20 μM siRNA stock solution and dilute to 125 μL (the final concentration added to the 6-well plate is about 12.5nM) for 5 minutes at room temperature. Dilute 10 μL of **EndoFectin™ RNAi** Transfection Reagent to 125 μL for 5 minutes at room temperature.

B. After 5 min, gently mix the diluted **siRNA** into the diluted **EndoFectin™ RNAi** transfection reagent (Note: Diluted **EndoFectin™ RNA**i transfection reagent should be mixed with the siRNA dilution within 30 minutes). Incubate at room temperature for 15 to 20 min to allow EndoFectin™ complexes to form.

3. Cells Transfection:

Add the **EndoFectin™** complex dropwise to the plate wells/dishes and gently shake the plate/dish during the dropping process to evenly expand the transfection reagent. Duplicate dropwise additions of transfection reagent to the same location should be avoided (it is easy to overly concentrate the local transfection reagent).

4. Cells Incubation and Analysis:

Incubate cells at 37°C in a 5% CO incubator and be ready for analysis after 24~72 hours. Due to the presence between different target genes and target cells, the most suitable detection time can be further explored.

■ Product Citation:

If use of this item results in a publication, please use this information: siRNA Oligos (Cat No. in the datasheet; GeneCopoeia, Inc., Rockville, MD).

Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of siRNA oligos (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged, or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product, or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.

GeneCopoeia, Inc.
9620 Medical Center Drive, #101, Rockville, MD 20850
Tel: 301-762-0888 Fax: 301-762-3888,
Email: inquiry@genecopoeia.com
Web: www.genecopoeia.com