

Protocol • EndoFectin™ RNAi Transfection Reagent • Catalog Nos. EF021/EF022

For efficient transfection of RNAi into mammalian cells

Description

EndoFectin™ RNAi transfection reagent is a transfection reagent based on the principle of lipofectamine® transfection. It is specially designed to deliver siRNA and miRNA to mammalian cells. It can form a complex with nucleic acid and allow the complex to enter the cell. EndoFectin™ RNAi transfection reagent is widely applicable to common cell lines, such as HEK-293, HEK293T, Hela, NIH/3T3 and A549, etc. This reagent efficiently delivers nucleic acids into cells even in the presence of serum.

EndoFectin™ RNAi provides the following advantages:

- Better transfection efficiency.
- Low cytotoxicity.
- For transfection of a variety of cell lines, easy to operate.
- Does not require removal of serum or culture medium, or removal of medium after transfection.

Contents and storage

Each vial contains 1 ml of sterile EndoFectin™ RNAi reagent.

EndoFectin™ RNAi is shipped with ambient temperature. Store the reagent at 4–8°C with the cap tightly closed. The reagent is stable for at least 12 months when stored at 4–8°C.

Before you start

Quality of siRNA

Purified siRNA by HPLC: Use high-quality transfection-grade siRNA or miRNA mimics, store them at an appropriate temperature (typically -20°C or -80°C) according to the siRNA instructions, and aliquot into small aliquots to avoid repeated freeze-thaw cycles.

Condition of cells

Always use high-quality cells that are well maintained and routinely authenticated which includes testing for bacteria, fungi, or Mycoplasma contamination. If the cells are from a recent liquid nitrogen stock, passage the cells at least 2 times before transfection.

Protocol for transient transfection

Materials:

- EndoFectin™ RNAi transfection reagent, siRNA, or miRNA
- Opti-MEM® I Reduced Serum Medium (Life Technologies. Catalog number: 31985-088).
- 50% confluence of cells

Before beginning a transfection experiment, we recommend first optimizing your transfection conditions with the EndoFectin™ RNAi transfection reagents. We suggest testing the amounts of EndoFectin™ RNAi transfection reagent listed in Table 1 (RNAi transfection) and Table 2 (RNAi and DNA co-transfection).

Table 1. Suggested starting conditions for RNAi transfection of adherent cells.

Culture vessel	Medium volume	Recommended dosage of mimics/siRNA	range of mimics/siRNA	dilution volume of mimics/siRNA/or EndoFectin RNAi	Recommended dosage of EndoFectin RNAi	range of EndoFectin RNAi
96-well plate (one well)	100 µL	1 pmol	0.5-4 pmol	5 µL	0.4 µL	0.2-0.8 µL
24-well plate (one well)	0.5 mL	5 pmol	2.5-20 pmol	25 µL	2 µL	1-4 µL
12-well plate (one well)	1.0 mL	10 pmol	5-40 pmol	50 µL	4 µL	2-8 µL
6-well plate (one well)	2.0 mL	25 pmol	12.5-100 pmol	125 µL	10 µL	5-20 µL
3.5-cm dish	2.0 mL	25 pmol	12.5-100 pmol	125 µL	10 µL	5-20 µL
6-cm dish	5.0 mL	75 pmol	37.5-300 pmol	375 µL	30 µL	15-60 µL

Table 2. Suggested starting conditions for RNAi and DNA co-transfection of adherent cells.

Culture vessel	Medium volume	Recommended dosage of DNA	Recommended dosage of mimics/siRNA	range of mimics/siRNA	dilution volume	Recommended dosage of EndoFectin RNAi	range of EndoFectin RNAi
96-well plate (one well)	100 µL	0.1 µg	5 pmol	2.5-10 pmol	5 µL	0.6 µL	0.4-1 µL
24-well plate (one well)	0.5 mL	0.5 µg	25 pmol	12.5-50 pmol	25 µL	3 µL	2-5 µL
12-well plate (one well)	1.0 mL	1 µg	50 pmol	25-100 pmol	50 µL	6 µL	4-10 µL
6-well plate (one well)	2.0 mL	2.5 µg	125 pmol	50-250 pmol	125 µL	15 µL	10-25 µL
3.5-cm dish	2.0 mL	2.5 µg	125 pmol	50-250 pmol	125 µL	15 µL	10-25 µL
6-cm dish	5.0 mL	7.5 µg	375 pmol	150-750 pmol	375 µL	45 µL	30-75 µL

For RNAi transfection

1. Cell plating culture: 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 be 50% confluent at the time of transfection.

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

2. Prepare RNAi-EndoFectin™ complexes:
 - A. Equilibrate RNAi, EndoFectin™ RNAi Transfection Reagent, and protein-free culture medium (e.g., Opti-MEM

EndoFectin™ RNAi Transfection Reagent User Manual

I™) to room temperature. Refer to Table 1 and dilute RNAi and EndoFectin™ RNAi transfection reagent in protein-free medium for 5 minutes at room temperature.

(For example: For siRNA transfection in 1 well of 6-well plate, dilute 12.5~100 pmol of siRNA to 125 µL for 5 minutes at room temperature. Dilute 5~20 µL of EndoFectin™ RNAi Transfection Reagent to 125 µL for 5 minutes at room temperature.

- B. After 5 min, gently mix the diluted RNAi into the diluted EndoFectin™ RNAi transfection reagent (Note: Diluted EndoFectin™ RNAi transfection reagent should be mixed with the RNAi dilution within 30 minutes). Incubate at room temperature for 15 to 20 min to allow RNAi-Transfection Reagent complexes to form.
3. Transfect the cells: 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. Incubate the cells and analyse:
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.

For RNAi and DNA co-transfection

1. Cell plating culture: 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 be 50% confluent at the time of transfection.

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

2. Prepare RNAi+DNA-EndoFectin™ complexes:
 - A. Equilibrate RNAi, DNA, EndoFectin™ RNAi Transfection Reagent, and protein-free culture medium (e.g., Opti-MEM I™) to room temperature. Refer to Table 2 and dilute RNAi+DNA and EndoFectin™ RNAi transfection reagent in protein-free medium for 5 minutes at room temperature.
(For example: For miRNA mimics and DNA co-transfection in 1 well of 6-well plate, dilute 50-250 pmol miRNA mimics+ 2.5 µg DNA to 125 µL for 5 minutes at room temperature. Dilute 10~25 µL of EndoFectin™ RNAi Transfection Reagent to 125 µL for 5 minutes at room temperature.
 - B. After 5 min, gently mix the diluted RNAi+DNA into the diluted EndoFectin™ RNAi transfection reagent (Note: Diluted EndoFectin™ RNAi transfection reagent should be mixed with the RNAi+DNA dilution within 30 minutes). Incubate at room temperature for 15 to 20 min to allow RNAi+DNA-Transfection Reagent complexes to form.
3. Transfect the cells: 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. Incubate the cells and analyse:
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.

Reminder

1. For cells that are sensitive to partial contact inhibition, the plating density can be appropriately reduced in Step 1 to reduce the confluence of the cells at the time of transfection.
2. The presence of serum has no negative effect on the transfection process, and RNAi-EndoFectin™ complexes can transfect cells smoothly even when the cell culture medium contains proteins (e.g., 10% serum). However, RNAi-EndoFectin™ complexes must be formed under protein-free conditions. Opti-MEM I™ medium was used to dilute RNAi and EndoFectin™ RNAi reagent for optimal transfection efficiency. If other protein-free products are used, the medium will need to be tested for compatibility with EndoFectin™ RNAi Transfection Reagent.

Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of EndoFectin™ RNAi Transfection Reagent (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.

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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