FT-DW9030

UptiFectin-OFF

Nanocarrier for siRNA Transfection

Gene silencing by siRNA transfection

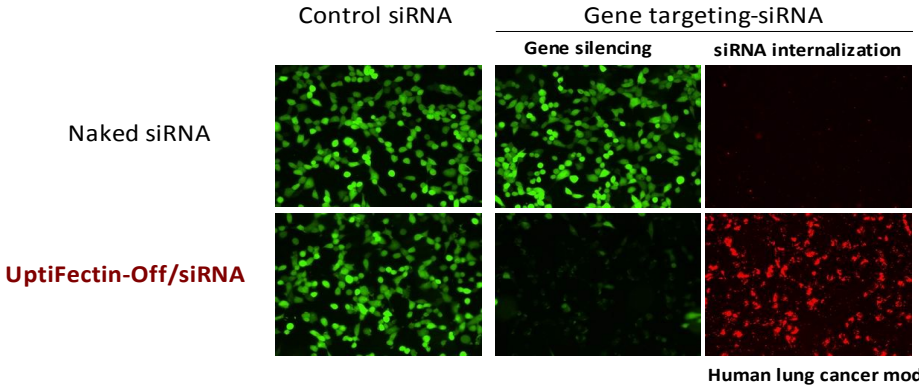
Product description

Name	Cat. Number	Quantity	
UptiFectin-OFF, Nanocarrier for siRNA Transfection			
	DW9030	0,5 ml	250 transfections in 24-well plates
	DW9031	4,0 ml	2000 transfections in 24-well plates
	DW903E	100 µl	50 transfections in 24-well plates

Storage +2 to +8°C.

Introduction

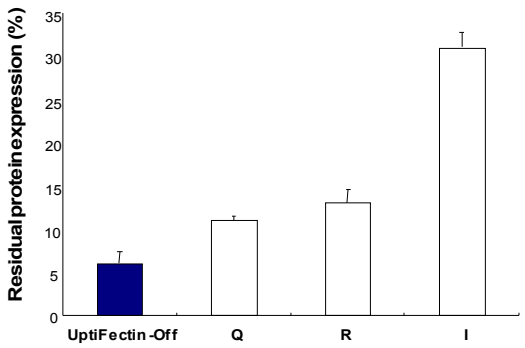
UptiFectin-OFF is a new synthetic derivative of a natural compound forming liposome in solution. **UptiFectin-OFF** is particulary suitable for primary and stem cell transfection with absence of toxicity at the effective concentration. **UptiFectin-OFF** / siRNA complex must be prepared in medium that does not contain serum (DMEM is recommended) even if cells are transfected in the presence of serum. Adherent cells are equally transfected either with forward or reverse transfection procedures. Suspension cells are transfected following the specific procedure described herein. Inhibition of the protein expression depends on the cell type, the nature of the protein and the amounts of **UptiFectin-OFF** and siRNA. Therefore transfection conditions should be optimized for every new cell type. Using in standard experimental conditions, 500µl of **UptiFectin-OFF** transfects siRNA over 250 wells in a 24-well format.



Human lung cancer model

Features

- Outstanding transfection efficiency for a wide variety of cell lines.
- Absence of toxicity at the effective concentrations.
- Removal of transfection complex is not needed.
- No need to keep complexes on ice during transfection.
- Easy handling.
- Excellent reproducibility.



High efficiency in comparison with other siRNA transfection reagents

For research purpose only. Not intended for animal or human therapeutic or diagnostic use.

Cell lines succefully tested with UptiFectin-OFF

HeLa, HEK 293, HepG2, MCF-7, HUVEC, HaCaT cells and many others

Technical and Scientific Information

1 Transfection of Adherent cells

1.1 Forward Transfection Procedure

Use the following procedure to transfect adherent cells in a 24-well format. For other formats, see **table 2** Scaling up/down Transfections.

1.2 Cell Preparation

One day before transfection, plate cells in 1 mL complete growth medium so that cells reach 70-80% confluence at the time of transfection ($0.5 – 2 \times 10^5$ cells per well).

1.2.1 UptiFectin-OFF/ siRNA complex preparation

All amounts and volumes are given to transfect one well in a 24-well format.

1. Thirty minutes before transfection, remove growth medium and add 500 µL fresh medium with or without serum (depending on the cells).
2. Dilute 37.5 ng (2.4 pmols) of siRNA (in a maximal volume of 5 µL H_2O) in 50 µL of DMEM without serum. Mix gently.
3. Vortex UptiFectin-OFF before use. Dilute 2 µL of UptiFectin-OFF in 50 µL of DMEM without serum. Mix gently.
4. Combine the diluted UptiFectin-OFF (52 µL) with the diluted siRNA (55 µL) by pipetting up and down five times and briefly vortexing.
5. Mix gently and incubate 15 minutes at room temperature.
6. Add the entire volume of complexes (107 µL) drop-wise to each well containing cells and 500 µL of fresh medium with or without serum (depending on the cells). Swirl the plate to ensure the homogeneous distribution of complexes.
7. Incubate cells with transfection complexes under their normal growth conditions until analysis. Medium may be supplemented with 50 µL serum 100% or changed (depending on the cells) after 2 to 4 hours if cells have been incubated without serum.

1.3 Reverse Transfection procedure

Use the following procedure to transfect siRNA the day of seeding cells in wells. Reverse transfection gains one day.

1.3.1 UptiFectin-OFF/ siRNA complex preparation

Amounts and volume are given to transfect one well in a 24-well format.

1. Dilute 37.5 ng (2.4 pmols) of siRNA (in a maximal volume of 5 µL H_2O) in 50 µL of DMEM without serum. Mix gently.
2. Vortex UptiFectin-OFF before use. Dilute 2 µL of UptiFectin-OFF in 50 µL of DMEM without serum. Mix gently.
3. Combine the diluted UptiFectin-OFF (52 µL) with the diluted siRNA (55 µL) by pipetting up and down five times and briefly vortexing.
4. Mix gently and incubate 15 minutes at room temperature.
5. Add the entire volume of complexes (107 µL) drop-wise to each well and then seed 1-4 x 10^5 cells per well in 500 µL culture medium with or without serum (depending on the cells). Swirl the plate to ensure the homogeneous distribution of cells.

Incubate cells with transfection complexes under their normal growth conditions until analysis. Medium may be supplemented with 50 µL serum 100% or changed (depending on the cells) after 2 to 4 hours if cells have been incubated without serum.

1.4 Optimizing transfection

To obtain the highest transfection efficiency, optimize transfection by varying siRNA quantity, UptiFectin-OFF amount and cell density.

For initial optimization in a 24-well format, use these nine UptiFectin-OFF/siRNA ratios (µL/ng).
Prepare complexes for a single well of a 24-well format as described in **table 1**.
Add the entire volume of complexes to each well.

Table 1: *Optimizing UptiFectin-OFF/siRNA ratio for adherent cell lines*

	siRNA amount in 5 µl H_2O		UptiFectin-OFF volume (µl)
Ratio	siRNA (ng)	siRNA (pmols)	
1/37.5	37.5	2.4**s**	1
2/37.5	37.5	2.4	**2**
4/37.5	37.5	2.4	4
1/75	75	4.8	1
2/75	75	4.8	2
4/75	75	4.8	4
1/100	100	6.4	1
2/100	100	6.4	2
4/100	100	6.4	4

1.5 Scaling up/down Transfections

To transfect cells in different cell culture formats, vary the amount of siRNA, UptiFectin-OFF, cells and medium used, according to **table 2** suggested proportions.

Culture format	Volume of plated cells	Volume of medium during transfection	DMEM volume in siRNA dilution tubes (µL)	Added siRNA (ng) in H_2O (µL) in siRNA dilution tubes	DMEM volume in UptiFectin-OFF dilution tubes (µL)	Added volume of UptiFectin-OFF to UptiFectin-OFF dilution tubes (µL)
96-well	200 µL	100 µL	10	7.5 ng in 1 µL	10	0.4
24-well	1 mL	500 µL	50	37.5 ng in 5 µL	50	2
12-well	2 mL	1 mL	100	75 ng in 10 µL	100	4
6-well	5 mL	2.5 mL	250	187.5 ng in 25 µL	250	10
35 mm	5 mL	2.5 mL	250	187.5 ng in 25 µL	250	10
60 mm	6 mL	3 mL	300	225 ng in 30 µL	300	12
100 mm	10 mL	5 mL	500	375 ng in 50 µL	500	20
T-25	5 mL	2.5 mL	250	187.5 ng in 25 µL	250	10
T-75	10 mL	5 mL	500	375 ng in 50 µL	500	20

Table 2: *Optimizing UptiFectin-OFF/siRNA ratio for adherent cell lines*To transfect cells in different cell culture formats, vary the amount of siRNA, UptiFectin-OFF, cells and medium used.

2 Transfection of suspension cells

2.1 Forward Transfection Procedure

Use the following procedure to transfect suspension cells in a 24-well format. For other formats, see Scaling up/down Transfections.

2.2 Cell preparation

Three hours before transfection, seed 2 x 10^5 cells per well in 200 µL of culture medium with or without serum (depending on the cells).

2.2.1 UptiFectin-OFF/ siRNA complex preparation

All amounts and volume are given to transfect one well in a 24-well format.

1. Dilute 375 ng (24 pmols) of siRNA (in a maximal volume of 5 µL H_2O) in 50 µL of DMEM without serum. Mix gently.
2. Vortex UptiFectin-OFF before use. Dilute 7 µL of UptiFectin-OFF in 50 µL of DMEM without serum. Mix gently.
3. Combine the diluted UptiFectin-OFF (57 µL) with the diluted siRNA (55 µL) by pipetting up and down five times and briefly vortexing.
4. Mix gently and incubate 15 minutes at room temperature.
5. Add the entire volume of complexes (112 µL) drop-wise to each well containing cells in 200 µL of medium with or without serum (depending on the cells). Swirl the plate to ensure the homogeneous distribution of complexes. Then, centrifuge the plates 5 minutes at 200g at room temperature.
6. Incubate the cells with the transfection complexes under their normal growth conditions for 3 hours.
7. Add 300 µL of culture medium containing serum to the cells whether cells were transfected in the presence or in the absence of serum and incubate until analysis.

2.3 Optimizing transfection

To obtain the highest transfection efficiency, optimize transfection by varying siRNA quantity, UptiFectin-OFF amount and cell density.

	siRNA amount in 5 µl H_2O		UptiFectin-OFF volume (µl)
Ratio	siRNA (ng)	siRNA (pmols)	
4/375	375	24	4
7/375	375	24	**7**
9/375	375	24	9
4/750	750	48	4
7/750	750	48	7
9/750	750	48	9
4/1000	1000	64	4
7/1000	1000	64	7
9/1000	1000	64	9

For initial optimization in a 24-well format, use these nine UptiFectin-OFF/siRNA ratios (µL/ng).
Prepare complexes for a single well of a 24-well format as described in table 3.
Add the entire volume of complexes to each well.

Table 3: *Optimizing UptiFectin-OFF/siRNA ratio for suspension cells.*

2.4 Scaling up/down Transfections

To transfect cells in different cell culture formats, vary the amount of siRNA, UptiFectin-OFF, cells and medium used according to **table 4** suggested proportions.

Culture formatl	Volume of plated cells	Number of plated cells	DMEM volume in siRNA dilution tubes (µL)	Added siRNA (ng) in H_2O (µL) in siRNA dilution tubes	DMEM volume in UptiFectin-OFF dilution tubes (µL)	Added volume of UptiFectin-OFF to UptiFectin-OFF dilution tubes (µL)	Volume of medium added after 3 hours
96-well	40 µL	4 x 10^4	10	75 ng in 1 µL	10	1.4	60 µL
24-well	200 µL	2 x 10^5	50	375 ng in 5µL	50	7	300 µL
12-well	400 µL	4 x 10^5	100	750 ng in 10 µL	100	14	600 µL
6-well	1 mL	10 x 10^5	250	1.875 µg in 25 µL	250	35	1.5 mL

Culture formatl	Volume of plated cells	Number of plated cells	DMEM volume in siRNA dilution tubes (µL)	Added siRNA (ng) in H_2O (µL) in siRNA dilution tubes	DMEM volume in UptiFectin-OFF dilution tubes (µL)	Added volume of UptiFectin-OFF to UptiFectin-OFF dilution tubes (µL)	Volume of medium added after 3 hours
35 mm	1 mL	10×10^5	250	1.875 µg in 25 µL	250	35	1.5 mL
60mm	1.2 mL	12×10^5	300	2.25 µg in 30 µL	300	42	1.8 mL
100 mm	2 mL	20×10^5	500	3.75 µg in 50 µL	500	70	3 mL
T-25	1 mL	10×10^5	250	1.875 µg in 25 µL	250	35	1.5 mL
T-75	2 mL	20×10^5	500	3.75 µg in 50 µL	500	70	3 mL

Table 4: *Optimizing UptiFectin-OFF/siRNA ratio for suspension cell lines*

Related Products

- UptiFectin-ON, Nanocarrier for DNA Transfection, DW9020
- Firefly Luciferase Assay Kit, *Bright Glow*, FP-JQ6811
- Firefly Luciferase 1-Step Assay Kit, FP-BX0320
- Firefly and Renilla Luciferase Assay Kit, FP-BE7810
- Coelenterazine H, R30783
- Coelenterazine (native), 972333
- X-Gal, 40534M
- Microplate Krystal 24 white TC treated 24 well plate, FP-BA8090
- Microplate Krystal 24 white TC treated 24 well plate with lid, FP-BA8120
- CelluFine Endotoxin Removal Mini Column, DW0610

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

UptiFectin reagent manufactured for Interchim by In-Cell-Art.

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