ICAFectin®442 : Reagent for siRNA transfection in primary and stem cells

Product description

- New synthetic derivative of natural compound.
- Particularly suitable for primary and stem cell transfection.
- Outstanding transfection efficiency for a wide variety of cell lines.
- Absence of toxicity at the effective concentrations.
- The ICAFectin®442/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.
- Removal of transfection complex is not needed.
- 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 ICAFectin®442 and siRNA. Therefore transfection conditions should be optimized for every new cell type.
- Storage at +2 to +8°C.
- No need to keep complexes on ice during transfection.
- Easy handling.
- Excellent reproducibility.
- Using standard experimental conditions, 500 µL of ICAFectin®442 transfects siRNA over 250 wells in a 24-well format.
- For research purpose only. Not intended for animal or human therapeutic or diagnostic use.

Important note for transfection : Do not include serum and antibiotics during the formation of the ICAFectin®442/siRNA complexes. For optimal transfection efficiency, we recommend DMEM for the ICAFectin®442/siRNA complex formation.

Transfection of adherent cells

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.

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 x 10^5 cells per well).

ICAFectin®442 / 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 150 ng (9.6 pmols) of siRNA (in a maximal volume of 5 µL H_2O) in 50 µL of DMEM without serum and antibiotics. Mix gently.
*Note : **Do not use** the serum-free culture medium in which cells were grown.*
3. Vortex ICAFectin®442 before use. Dilute 2 µL of ICAFectin®442 in 50 µL of DMEM without serum and antibiotics. Mix gently.
*Note : **Do not use** the serum-free culture medium in which cells were grown.*
4. Combine the diluted ICAFectin®442 (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.

Reverse Transfection procedure

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

ICAFectin®442 / siRNA complex preparation

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

1. Dilute 150 ng (9.6 pmols) of siRNA (in a maximal volume of 5 µL H_2O) in 50 µL of DMEM without serum and antibiotics. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown.*

2. Vortex ICAFectin®442 before use. Dilute 2 µL of ICAFectin®442 in 50 µL of DMEM without serum and antibiotics. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown.*

3. Combine the diluted ICAFectin®442 (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.
6. 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.

Optimizing transfection

To obtain the highest transfection efficiency, optimize transfection by varying siRNA quantity, ICAFectin®442 amount and cell density.

	siRNA amount in 5 µl H_2O		ICAFectin®442 volume (µl)
Ratio (µl/ng)	siRNA (ng)	siRNA (pmols)	
1/75	75	4.8	1
2/75	75	4.8	**2**
4/75	75	4.8	4
1/150	150	9.6	1
2/150	150	9.6	2
4/150	150	9.6	4
1/300	300	19.2	1
2/300	300	19.2	2
4/300	300	19.2	4

***Table 1:** Optimizing ICAFectin®442/siRNA ratio for adherent cell lines*

For initial optimization in a 24-well format, use these nine ICAFectin®442 /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.

Scaling up/down Transfections

To transfect cells in different cell culture formats, vary the amount of siRNA, ICAFectin®442, 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 ICAFectin®442 dilution tubes (µL)	Added volume of ICAFectin®442 to ICAFectin®442 dilution tubes (µL)
96-well	200 µL	100 µL	10	30 ng in 1 µL	10	0.4
24-well	1 mL	500 µL	50	150 ng in 5 µL	50	2
12-well	2 mL	1 mL	100	300 ng in 10 µL	100	4
6-well	5 mL	2.5 mL	250	750 ng in 25 µL	250	10
35 mm	5 mL	2.5 mL	250	750 ng in 25 µL	250	10
60 mm	6 mL	3 mL	300	900 ng in 30 µL	300	12
100 mm	10 mL	5 mL	500	1500 ng in 50 µL	500	20
T-25	5 mL	2.5 mL	250	750 ng in 25 µL	250	10
T-75	10 mL	5 mL	500	1500 ng in 50 µL	500	20

Table 2: *Optimizing ICAFectin®442/siRNA ratio for adherent cell lines*

Transfection of suspension cells

Forward Transfection Procedure

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

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).

ICAFectin®442 / 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 and antibiotics. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown.*

2. Vortex ICAFectin®442 before use. Dilute 7 µL of ICAFectin®442 in 50 µL of DMEM without serum and antibiotics. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown.*

3. Combine the diluted ICAFectin®442 (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.

Optimizing transfection

To obtain the highest transfection efficiency, optimize transfection by varying siRNA quantity, ICAFectin®442 amount and cell density.

Ratio (µl/ng)	siRNA amount in 5 µl H_20		ICAFectin®442 volume (µl)
	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 ICAFectin®442/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 ICAFectin®442/siRNA ratio for suspension cells.*

Scaling up/down Transfections

To transfect cells in different cell culture formats, vary the amount of siRNA, ICAFectin®442, cells and medium used according to **table 4** suggested proportions.

Culture format	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 ICAFectin®442 dilution tubes (µL)	Added volume of ICAFectin®442 to ICAFectin®442 dilution tubes (µL)	Volume of medium added after 3 hours
96-well	40 µL	4×10^4	10	75 ng in 1 µL	10	1.4	60 µL
24-well	200 µL	2×10^5	50	375 ng in 5µL	50	7	300 µL
12-well	400 µL	4×10^5	100	750 ng in 10 µL	100	14	600 µL
6-well	1 mL	10×10^5	250	1 875 µg in 25 µL	250	35	1.5 mL
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	2 250 µg in 30 µL	300	42	1.8 mL
100 mm	2 mL	20×10^5	500	3 750 µg in 50 µL	500	70	3 mL
T-25	1 mL	10 x 105	250	1 875 µg in 25 µL	250	35	1.5 mL
T-75	2 mL	20 x 105	500	3 750 µg in 50 µL	500	70	3 mL

Table 4: *Optimizing ICAFectin®442/siRNA ratio for suspension cell lines*