ICAFectin®441: Reagent for DNA 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®441/DNA complex must be prepared in medium that does not contain serum (Opti MEM 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.
- ICAFectin®441 can complex in the same particle DNA and siRNA molecules, thus allowing gene expression and inhibition in the same experiment.
- Expression of the transfected gene depends on the cell type, the promoter used, the nature of the expressed protein and the amounts of ICAFectin®441 and DNA. Therefore transfection conditions should be optimized for every new cell type.
- No need to keep complexes on ice during transfection.
- Storage at +2 to +8°C.
- Easy handling.
- Excellent reproducibility.
- Using standard experimental conditions, 500 µL of ICAFectin®441 transfects over 650 wells in a 24-well format.
- For research purposes 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®441/DNA complexes. For optimal transfection efficiency, we recommend Opti MEM I for the ICAFectin®441/DNA 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®441 / DNA 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 0.5 µg of DNA (in a maximal volume of 5 µL H2O) in 50 µL of Opti-MEM I without serum. Mix gently.

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

3. Vortex ICAFectin®441 before use. Dilute 0.75 µL of ICAFectin®441 in 50 µL of Opti-MEM without serum. Mix gently.

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

4. Combine the diluted ICAFectin®441 (50.75 µL) with the diluted DNA (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 (105.75 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 condition growth 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.

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Reverse Transfection procedure

Use the following procedure to transfect DNA the day of seeding cells in wells. Reverse transfection gains one day. Amounts and volume are given to transfect one well in a 24-well format.

ICAFectin®441 / DNA complex preparation

1. Dilute 0.5 µg of DNA (in a maximal volume of 5 µL H2O) in 50 µL of Opti-MEM without serum. Mix gently.

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

2. Vortex ICAFectin®441 before use. Dilute 0.75 µL of ICAFectin®441 in 50 µL of Opti-MEM without serum. Mix gently.

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

3. Combine the diluted ICAFectin®441 (50.75 µL) with the diluted DNA (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 (105.75 µL) drop-wise to each well and then seed 1-4 x 10^5 cells per well in 500 µL of fresh 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.

Cotransfection of DNA and siRNA procedure

Use the following procedure for forward and reverse transfection of DNA and siRNA in a 24-well format. Amounts and volumes are given to transfect one well in a 24-well format.

ICAFectin®441/ DNA and siRNA complex preparation

1. Dilute 0.5 µg of DNA and 75 ng (4.8 pmols) of siRNA (in a maximal volume of 5 µL H2O) in 50 µL of Opti-MEM without serum. Mix gently.

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

2. Vortex ICAFectin®441 before use. Dilute 0.75 µL of ICAFectin®441 in 50 µL of Opti-MEM without serum. Mix gently.

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

3. Combine the diluted ICAFectin®441 (50.75 µL) with the diluted DNA and 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 (105.75 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.
6. Incubate cells with transfection complexes under their normal growth conditions until analysis. Medium may be supplemented with 50µL 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, transfect in medium that does not contain serum and optimize transfection by varying DNA quantity, ICAFectin®441 amount and cell density.

For initial optimization in a 24-well format, use these six ICAFectin®441/DNA ratios (µL/µg).

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.

Ratio (µL/µg)	DNA amount (µg) in 5µL H_2O	ICAFectin®441 volume (µL)
0.5/0.5	0.5	0.5
0.75/0.5	**0.5**	**0.75**
1.3/0.5	0.5	1.3
1/1	1	1.0
1.5/1	1	1.5
2.6/1	1	2.6

***Table 1:** Optimizing ICAFectin®441/DNA ratio for adherent cell lines*

Scaling up/down Transfections

To transfect cells in different cell culture formats, vary the amount of DNA, ICAFectin®441, cells and medium used, according to **table 2** suggested proportions.

Culture format	Volume of plated cells	Volume of medium during transfection	Opti-MEM volume in DNA dilution tubes (µL)	Added DNA (µg) in H_2O (µL) in DNA dilution tubes	Opti-MEM volume in ICAFectin®441 dilution tubes (µL)	Added volume of ICAFectin®441 to ICAFectin®441 dilution tubes (µL)
96-well	200 µL	100 µL	10	0.1 µg in 1 µL	10	0.15 µL
24-well	1 mL	500 µL	50	0.5 µg in 5µL	50	0.75 µL
12-well	2 mL	1 mL	100	1 µg in 10 µL	100	1.5 µL
6-well	5 mL	2.5 mL	250	2.5 µg in 25 µL	250	3.8 µL
35 mm	5 mL	2.5 mL	250	2.5 µg in 25 µL	250	3.8 µL
60mm	6 mL	3 mL	300	3 µg in 30 µL	300	4.6 µL
100 mm	10 mL	5 mL	500	5 µg in 50 µL	500	7.6 µL
T-25	5 mL	2.5 mL	250	2.5 µg in 25 µL	250	3.8 µL
T-75	10 mL	5 mL	500	5 µg in 50 µL	500	7.6 µL

Table 2: *Optimizing ICAFectin®441/DNA 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®441/DNA complex preparation

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

1. Dilute 1 µg of DNA (in a maximal volume of 5 µL H2O) in 50 µL of Opti-MEM I without serum. Mix gently.

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

2. Vortex ICAFectin®441 before use. Dilute 1.5 µL of ICAFectin®441 in 50 µL of Opti-MEM without serum. Mix gently.

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

3. Combine the diluted ICAFectin®441 (51.5 µL) with the diluted DNA (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 (106.5 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, transfect with medium that does not contain serum and after 3 hours add 300 µL of culture medium containing serum to the cells. You can also optimize transfection by varying DNA quantity, ICAFectin®441 amount and cell density.

For initial optimization in 24-well format, use these six ICAFectin®441/DNA ratios (µL/µg).

Prepare complexes for a single well of a 24-well format as described in **table 3**.

Ratio (µL/µg)	DNA amount (µg) in 5µL H_2O	ICAFectin®441 volume (µL)
1/1	1	1
1.5/1	1	1.5
2.6/1	1	2.6
2/2	2	2
3/2	2	3
5.2/2	2	5.2

Table 3: *Optimizing ICAFectin®441/DNA ratio for suspension cells.*

Scaling up/down Transfections

To transfect cells in different cell culture formats, vary the amount of DNA, ICAFectin®441, cells and medium used according to **table 4** suggested proportions.

Culture format	Volume of plated cells	Number of plated cells	Opti-MEM volume in DNA dilution tubes (µL)	Added DNA (µg) in H_2O (µL) in DNA dilution tubes	Opti-MEM volume in ICAFectin®441 dilution tubes	Added volume of ICAFectin®441 to ICAFectin®441 dilution tubes	Volume of medium added after 3 hours
96-well	40 µL	4×10^4	10 µL	0.2 µg in 1 µL	10 µL	0.3 µL	60 µL
24-well	200 µL	2×10^5	50 µL	1 µg in 5µL	50 µL	1.5 µL	300 µL
12-well	400 µL	4×10^5	100 µL	2 µg in 10 µL	100 µL	3 µL	600 µL
6-well	1 mL	10×10^5	250 µL	5 µg in 25 µL	250 µL	7.6 µL	1.5 mL
35 mm	1 mL	10×10^5	250 µL	5 µg in 25 µL	250 µL	7.6 µL	1.5 mL
60mm	1.2 mL	12×10^5	300 µL	6 µg in 30 µL	300 µL	9.2 µL	1.8 mL
100 mm	2 mL	20×10^5	500 µL	10 µg in 50 µL	500 µL	15.2 µL	3 mL
T-25	1 mL	10×10^5	250 µL	5 µg in 25 µL	250 µL	7.6 µL	1.5 mL
T-75	2 mL	20×10^5	500 µL	10 µg in 50 µL	500 µL	15.2 µL	3 mL

Table 4: *Optimizing ICAFectin®441/DNA ratio for suspension cell lines*