
jetSI™-ENDO *in vitro* siRNA and DNA/siRNA co-transfection Transfection Protocol

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Company Information

Technical Assistance and Scientific Advice

Contact the friendly Polyplus technical support *via*:

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Trademarks

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We recommend the use of INTERFERin™ siRNA Transfection Reagent for the transfection of siRNA duplexes at low siRNA concentrations.

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Product Information

jetSI™-ENDO siRNA transfection reagent provides efficient gene silencing and reproducible transfection of mammalian cells with low toxicity. jetSI™-ENDO is synthesized and purified at Polyplus-transfection. jetSI™-ENDO is especially developed to efficiently deliver siRNA duplexes into mammalian cells. It is also particularly well suited for co-transfection experiment using siRNA and plasmid DNA. jetSI™-ENDO compacts siRNA duplexes and plasmid DNA into positively charged particles capable of interacting with anionic proteoglycans at the cell surface and entering cells by endocytosis. It protects siRNA duplexes and plasmid DNA from degradation and favors rapid endosomal release into the cytoplasm.

Publications using jetSI™-ENDO can be searched on the Product Citation Database. The Cell Transfection Database gives transfection conditions for over 400 cell lines and primary cells. Both databases are available on the Polyplus website, www.polyplus-transfection.com, under the Products/Technical Resources heading.

Ordering information

Cat #	Reagent Size	Number of transfections
402-10	1 ml	1000 siRNA transfections in 24-well plates at 50 nM siRNA

Content

1 ml of jetSI™-ENDO transfection reagent is sufficient to perform 600 co-transfections in 24-well plates or 1000 transfections (using 50 nM of siRNA)

Reagent use and Limitations

For research use only. Not for use in humans.

Quality control

Functional analysis: every batch of jetSI™-ENDO is tested in a co-transfection assay on HeLa cells in the presence of serum. The silencing efficiency obtained for each batch is indicated on the Certificate of Analysis.

Formulation and Storage

jetSI™-ENDO should be stored tightly capped at 4°C upon arrival. **Do not freeze. Always ensure the tube is tightly closed after use to avoid evaporation.** jetSI™-ENDO is stable for at least 6 months at 4°C, as indicated on the Certificate of Analysis.

1. Co-transfection of siRNA and plasmid DNA

1.1. Cell seeding

For optimal transfection of adherent cells with jetSI™-ENDO, cells should be seeded the day before transfection at 25-40% confluency (refer to Table 1 for the number of cells to seed according to the culture vessel format). For optimal transfection of suspension cells with jetSI™-ENDO, cells are seeded on the day of transfection with the number of cells recommended in Table 1.

Table 1. Recommended number of cells to seed

Culture vessel	Number of adherent cells to seed 1 day before transfection	Number of suspension cells to seed on the day of transfection	Surface area per well or plate (cm ²)	Volume of medium per well or plate
96-well	5 000 ⁽ - 10 000 ⁽	20 000 ⁽ - 50 000 ⁽	0.3	0.2 ml
48-well	10 000 [*] - 20 000 ^{**}	50 000 [*] - 200 000 ^{**}	1	0.5 ml
24-well	20 000 [*] - 40 000 ^{**}	200 000 [*] - 1 000 000 ^{**}	1.9	1 ml
12-well	30 000 [*] - 80 000 ^{**}	500 000 [*] - 2 000 000 ^{**}	3.8	2 ml
6-well/ 35 mm	100 000 [*] - 200 000 ^{**}	1 000 000 [*] - 4 000 000 ^{**}	9.4	4 ml
6 cm	200 000 [*] - 400 000 ^{**}	/	28	8 ml
10 cm	500 000 [*] - 1 000 000 ^{**}	/	78.5	10 ml

() Number of cells recommended when gene silencing is assessed 2 to 4 days after transfection.

(^{}) Number of cells recommended when gene silencing is assessed 24 hours post-transfection.*

1.2. Preparation of complexes and transfection procedure in presence of serum

- Check the concentration of the siRNA duplexes (even if provided by the manufacturer)
- Use RNase and pyrogen free materials (Tips, tubes, low binding microtubes, solution)

The following protocol is for co-transfection of **140 ng of siRNA duplexes** (20 nM) and **400 ng of plasmid DNA** per well in a **24-well plates**. Refer to Table 2 for transfection in other culture formats.

1. Per well, dilute 1.6 µl of jetSI™-ENDO into 50 µl of serum-free medium. Vortex vigorously (important: do not pipet to mix) and incubate at room temperature for 10 min (do not exceed 30 min).
2. Per well, dilute 140 ng of siRNA duplexes and 400 ng of plasmid DNA into 50 µl of serum-free medium. Vortex gently.
3. Add the 50 µl jetSI™-ENDO solution to the 50 µl siRNA/plasmid DNA solution all at once (do not mix the solutions in the reverse order).
4. Immediately vortex-mix the solution for 10 seconds.
5. Incubate for 15 min at room temperature (do not exceed 30 min).
6. In the meantime, replace cell medium with 0.5 ml of pre-warmed growth medium.
7. Add the 100µl jetSI™-ENDO/siRNA:DNA mix into each well and homogenize the medium by gently swirling the plate.
8. After 4 h of incubation, add 0.5 ml of medium containing serum per well and return the plate to the incubator.
9. Gene expression is usually measured 24 h to 96 h post-transfection.

Table 2: Co-transfection conditions according to the cell culture formats at 20 nM siRNA final

Culture format	siRNA amount per well	DNA amount per well	Volume of serum-free medium for siRNA and jetSI-ENDO™ dilution	Volume of jetSI™-ENDO reagent	Complete growth medium on cells	Volume of medium to add after 4 h
96-w	28 ng	120 ng	10 µl	0.45 µl	0.1 ml	0.1 ml
24-w	140 ng	0.4 µg	50 µl	1.6 µl	0.5 ml	0.5 ml
12-w	280 ng	0.8 µg	50 µl	3.2 µl	1 ml	1 ml
6-w/3 cm	560 ng	1.6 µg	100 µl	6.4 µl	2 ml	2 ml

2. Transfection of siRNA for gene silencing

2.1. Cell seeding

The number of cells to seed is the same as when performing co-transfection (refer to section 1.1 and Table 1)

2.2. Preparation of complexes and transfection procedure

- *Check the concentration of the siRNA duplexes (even if provided by the manufacturer)*
- *Use RNase and pyrogen free materials (Tips, tubes, low binding microtubes, solution)*

In order to optimize endogenous gene silencing, we recommend testing siRNA concentrations ranging from **10 to 50 nM**.

2.2.1. Preparation of complexes and transfection procedure in presence of serum

The following protocol is given for the transfection of 70 ng siRNA duplexes per well (10 nM) in 24-well plates (see Table 3 for transfection conditions in other culture formats).

- 1 - Per well, dilute 1 µl of jetSI™-ENDO in 50 µl of medium without serum. Vortex vigorously (important: do not pipet to mix) and incubate at room temperature for 10 min (do not exceed 30 minutes).
- 2 - Per well, dilute 70 ng of siRNA duplexes in 50 µl of medium without serum. Vortex gently.
- 3 - Add the 50 µl jetSI™-ENDO solution to the 50 µl siRNA solution all at once (do not mix the solutions in the reverse order).
- 4 - Immediately vortex-mix the solution for 10 seconds.
- 5- Incubate for 15 minutes at room temperature (do not exceed 30 min).
- 6 - In the meantime, replace cell medium with 0.5 ml of pre-warmed growth medium.
- 7 - Add the 100 µl jetSI™-ENDO/siRNA mix into each well and homogenize by gently swirling the plate.
- 8 - After 4 h of incubation, add 0.5 ml of medium containing serum per well and return the plate to the incubator.
- 9 - Gene expression is usually measured at 24 h to 96 h post-transfection

Table 3. Recommended transfection conditions in various cell culture formats at 10 nM siRNA

Culture format	siRNA duplexes (pmoles)*	Amount of siRNA per well*	Volume of serum-free medium for siRNA and jetSI™-ENDO dilution	Volume of jetSI™-ENDO reagent	Complete growth medium on cells	Medium to add after 4 h
96-w	1	14 ng	10 µl	0.4 µl	0.1 ml	0.1 ml
24-w	5	70 ng	50 µl	1 µl	0.5 ml	0.5 ml
12-w	10	140 ng	50 µl	2 µl	1 ml	1 ml
6-w/35mm	20	280 ng	100 µl	4 µl	2 ml	2 ml
6 cm	40	560 ng	200 µl	7 µl	4 ml	4 ml
10 cm	50	700 ng	250 µl	10 µl	5 ml	5 ml

*siRNA amount should be optimized for your own use and siRNA final concentration should range between 10 and 50 nM for optimal results.

If the volumes of jetSI™-ENDO to pipet are less than 0.5 µl, jetSI™-ENDO may be diluted just before use in ethanol (e.g. 1/2 or 1/10).

2.2.2. Preparation of complexes and transfection procedure in absence of serum

The experiment can be performed in absence of serum, if needed. Please proceed as previously (see 2.2.1) until step 5, then proceed as follow:

6' - During complex formation, remove medium from the plates and add 0.5 ml of serum-free medium, pre-warmed at 37°C.

7' - Add the 100 µl jetSI™-ENDO/siRNA mix into the serum-free medium in each well and homogenize by gently swirling the plate.

8' - Incubate the plate at 37°C in humidified atmosphere with 5% CO₂ for 4 hours

9' - Add 0.5 ml of medium containing 2 X concentrated serum (e.g. add 20% of serum when final serum concentration is meant to be 10%) and incubate the plate as before.

10' - Gene expression is usually measured at 24 h to 96 h post-transfection.

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Troubleshooting

Observations	Actions
Low transfection efficiency	<ul style="list-style-type: none"> ▪ Optimize the amount of siRNA used in the transfection assay. ▪ Use high-quality siRNA (PAGE purified and desalted). ▪ Ensure that adherent cells are 30-50% confluent at the time of transfection. ▪ Optimize the jetSI™-ENDO/siRNA duplexes ratio (volume wise). ▪ Decrease the volume of medium used per well.
Cellular toxicity	<ul style="list-style-type: none"> ▪ Decrease the amount of siRNA used in the transfection assay (keeping the jetSI™-ENDO/siRNA duplexes ratio constant). ▪ Reduce the incubation time of jetSI™-ENDO/siRNA complexes with the cells (e.g. 2 to 4 hours). ▪ Verify that silencing of the target expression is not affecting cell viability.