jetSI™-ENDO

Cationic polymer transfection reagent

In vitro siRNA Transfection Protocol

jetSI™-ENDO	Cat. N° 402-04	0.4 ml	(400 siRNA transfections in 24-well plates, at 50 nM siRNA)
jetSI™-ENDO	Cat. N° 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 ca. 1000 transfections (using 50 nM of siRNA) in 24-well plates. For research use only.

Storage

jetSI™-ENDO should be stored at **4°C** upon arrival (tightly capped). **Always make sure to tightly close the tube after each use to avoid ethanol evaporation.** jetSI™-ENDO is stable for 6 months at 4°C. **Do not freeze.**

Description

jetSI™-ENDO is a powerful siRNA transfection reagent that ensures efficient gene silencing and reproducible transfection with low toxicity. jetSI™-ENDO is synthesized and purified by PolyPlus-transfection. jetSI™-ENDO is specially developed to efficiently deliver siRNA duplexes to mammalian cells in presence of serum. jetSI™-ENDO compacts siRNA duplexes into positively charged particles capable of interacting with anionic proteoglycans at the cell surface and entering cells by endocytosis. Its properties protect siRNA duplexes from degradation and favor rapid endosomal release into the cytoplasm.

Quality control

Functional analysis: every batch of jetSI™-ENDO is tested in house by endogenous silencing on A549-GL3-Luc cells.

Ref : CPT 402
Version : F

General considerations

RNA interference (RNAi) is a new method for gene silencing in mammalian cells[1-3]. Optimized short RNA duplexes (siRNA; small interfering RNA) appear to be extremely selective by interacting with a single target in the mRNA, providing sequence-specific mRNA degradation and inhibition of protein production. When siRNA duplexes are introduced by transfection, RNAi has been shown to effectively silence endogenous genes in a variety of mammalian cells.

Transfection Protocols

Transfection of siRNA for endogenous gene silencing

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 recommended number of cells to seed according to the culture vessel formats). For optimal transfection of suspension cells with jetSI™-ENDO, cells are seeded on the day of transfection with the recommended number of cells according to the culture vessel formats (see 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.

2. Preparation of complexes and transfection procedure

Notes:

- *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 a range of siRNA concentrations from 1 nM to 50 nM.

2. 1. Preparation of complexes and transfection procedure in the presence of serum

The following protocol is for transfection of siRNA duplexes at 70 ng/well (10 nM) in one well of a 24-well plates, see Table 2 for transfection in other culture formats and table 3 for transfection with different siRNA concentration.

• For each well, dilute 1 µl of jetSI™-ENDO solution into 50 µl of serum-free medium. Vortex **vigorously (important: do not pipet to mix)** and wait for 10 min **(important: do not exceed 30 minutes)**.

• For each well, dilute 70 ng of siRNA duplexes into 50 µl of serum-free medium. Vortex gently.

• Add the 50 µl jetSI™-ENDO serum-free medium solution to the 50 µl siRNA duplexes solution all at once (**important: do not mix the solutions in the reverse order**).

• **Immediately** vortex-mix the solution for 10 seconds.

• Incubate for **15 minutes at room temperature** to allow complexes to form **(important: do not exceed 30 min)**.

• During complex formation, remove the growth medium from the plates and add 0.5 ml of fresh serum-containing medium, pre-warmed at 37°C.

• Add the 100µl jetSI™-ENDO /siRNA duplexes mixture into each well and homogenize the mixture by gently swirling the plate.

• After 4 h of incubation, complete volume up to 1 ml with serum-containing medium and incubate the plate under the required conditions.

• Gene expression is measured at suitable time points (usually between 24 h - 96 h)

Table 2 . Transfection conditions

Culture format	siRNA duplexes (stock at 20 µM)	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	siRNA concentration during the 4h transfection*	Medium added after 4 h
96-w	1 pmoles	14 ng	10 µl	0.4 µl	0.1 ml	10 nM*	0.1 ml
24-w	5 pmoles	70 ng	50 µl	1 µl	0.5 ml	10 nM*	0.5 ml
12-w	10 pmoles	140 ng	50 µl	2 µl	1 ml	10 nM*	1 ml
6-w	20 pmoles	280 ng	100 µl	4 µl	2 ml	10 nM*	2 ml
6 cm	40 pmoles	560 ng	200 µl	7 µl	4 ml	10 nM*	4 ml
10 cm	50 pmoles	700 ng	250 µl	10 µl	5 ml	10 nM*	5 ml

** siRNA concentration should be optimized for your own use*

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

Table 3 . Amount of jetSI™-ENDO according to culture format and siRNA concentration

Culture format	siRNA concentration during the 4h transfection	Volume of jetSI™-ENDO reagent	Final siRNA/jetSI™-ENDO complexes volume	Complete growth medium on cells
96-w	From 1 to 50 nM	0.4 µl	20 µl	0.1 ml
24-w	From 1 to 50 nM	1 µl	100 µl	0.5 ml
12-w	From 1 to 50 nM	2 µl	100 µl	1 ml
6-w	From 1 to 50 nM	4 µl	200 µl	2 ml
6 cm	From 1 to 50 nM	7 µl	400 µl	4 ml
10 cm	From 1 to 50 nM	10 µl	500 µl	5 ml

* Note that volume of jetSI™-ENDO recommended to use is independent of siRNA concentration.

2. 2. Preparation of complexes and transfection procedure in the absence of serum

The following protocol is for transfection of siRNA duplexes at 70 ng/well (10 nM) in one well of a 24-well plate, see Table 2 for transfection in other culture formats and table 3 for transfection with different siRNA concentration.

• For each well, dilute 1 µl of jetSI™-ENDO solution into 50 µl of serum-free medium. Vortex **vigorously (important: do not pipet to mix)** and wait for 10 min **(important: do not exceed 30 minutes)**.

• For each well, dilute 70 ng of siRNA duplexes into 50 µl of serum-free medium. Vortex gently.

• Add the 50 µl jetSI™-ENDO serum-free medium solution to the 50 µl siRNA duplexes solution all at once (**important: do not mix the solutions in the reverse order**).

• **Immediately** vortex-mix the solution for 10 seconds.

• Incubate for **15 minutes at room temperature** to allow complexes to form **(important: do not exceed 30 min)**.

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

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

• Incubate the plate under the required cell culture conditions for 4 h, then 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.

• Gene expression is measured at suitable time points (usually between 24 h - 96 h)

Factors affecting transfection efficiency

• Usually, transfection efficiencies can be improved by using smaller volumes of medium.

• For fragile cells, the transfection complexes can be removed after 4 h incubation. For this, aspirate the medium containing the complexes and replace it with fresh 1 X concentrated serum containing medium.

Ref : CPT 402
Version : F

Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	• 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 the day of transfection. • Optimize the jetSI™-ENDO /siRNA duplexes ratio (volume wise). • Decrease the culture medium volume.
Cellular toxicity	• 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.

Technical Assistance

Contact the PolyPlus assistance service via:
Internet address: www.polyplus-transfection.com
Email: support@polyplus-transfection.com
Telephone: + 33 (0) 3 90 40 61 87

Related compounds for transfection

jetSI™-ENDO-FluoF, Cat N° 407-04
jetSI™-ENDO-FluoR, Cat N° 408-04

jetPEI™ and derivatives for both *in vitro* and *in vivo* gene delivery applications.

References

1. McManus,MT *et al.* (2002) Gene silencing in mammals by small interfering RNAs. Nature Reviews Genetics **3**: 737-747
2. Hannon GJ. (2002) RNA interference. Nature **418**: 244-251
3. Elbashir, SM *et al.* (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in mammalian cell culture. Nature **411**: 494-498.

Ref : CPT 402
Version : F

NOTES