jetPRIME® transfection reagent

Short protocol – siRNA transfection

RNA INTERFERENCE

Day 0: Cell seeding

→ Seed cells in **V** mL of cell growth medium according to the table below

Quantities per well, dish or flask

Culture vessel	Number of cells *	V = volume of growth medium for cell seeding
24-well	25 000 - 40 000	0.5 mL
12-well	50 000 - 80 000	1 mL
6-well / 35 mm	100 000 - 150 000	2 mL
100 mm / flask 75 cm^2	0.5 x 10^6 - 1 x 10^6	10 mL

*For specific cell type or suspension cells, please refer to the complete protocol.

Day 1: Transfection

→ Perform transfection **in the presence of serum**

→ Use **jetPRIME® buffer only**

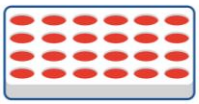
→ Transfect cells at **50% confluency**

Dilute **X** pmoles of siRNA in **W** µL of **jetPRIME® buffer**. Vortex 10 s and spin down

Add **Y** µL of **jetPRIME® reagent**

Vortex 1 s, spin down and incubate 10 min at RT

Add transfection mix to the cells in serum containing medium



Incubate 24 to 72 h

Quantities per well, dish or flask

Culture vessel	W = volume of jetPRIME® buffer	X = amount of siRNA added (10 nM)	X = amount of siRNA added (50 nM)	Y = volume of jetPRIME® reagent
24-well	50 µL	5.5 pmoles (76 ng)	27.5 pmoles (381 ng)	2 µl
12-well	100 µL	11 pmoles (152 ng)	55 pmoles (762 ng)	3 µl
6-well / 35 mm	200 µL	22 pmoles (306 ng)	110 pmoles (1524 ng)	4 µl
100 mm / flask 75 cm^2	500 µL	105 pmoles (1460 ng)	525 pmoles (7274 ng)	20 µl

Day 2-3: Measure gene expression

See back page for optimization tips

Download complete protocol on https://myaccount.polyplus-transfection.com/

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jetPRIME® transfection reagent

Short protocol – Optimization tips (siRNA)

RNA INTERFERENCE

Protocol Optimization

- Test different siRNA concentration ranging from 10 to 50 nM (final concentration)
- Use cells at 50% confluency at time of transfection.
- For cell specific protocols, check our online Cell Transfection Database:

http://www.polyplus-transfection.com/resources/cell-transfection-database/

Tips to increase cell viability of sensitive cells

- Replace medium 4 h after transfection.
- Check that silencing the target gene does not affect cell viability.

Use appropriate controls

- Positive control: siRNA against housekeeping genes/fluorescently labelled siRNA.
- Negative control: mismatch, scramble or non-targeting sequence.

Good siRNA Transfection Practices

- Store appropriately jetPRIME® (5 ± 3°C).
- Ensure that cells have been passaged more than twice and less than 20 times prior to transfection.
- Discard overconfluent cells.
- Check the half-lives of the protein and mRNA of interest and measure gene silencing accordingly 24 to 96 h after transfection.
- Regularly check for mycoplasma contaminations.
- Serum quality may drastically affect transfection efficiency. When purchasing a new batch of serum or trypsin, check cell viability as well as transfection efficiency.

Note: jetPRIME® is also recommended for DNA transfection and DNA/siRNA co-transfection. Please refer to the complete protocol available when creating your account online at: https://myaccount.polyplus-transfection.com/.

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