jetPRIME® transfection reagent
Short protocol – siRNA Transfection

DAY 0: Cell seeding

→ Seed cells in V mL of serum containing medium according to the table below

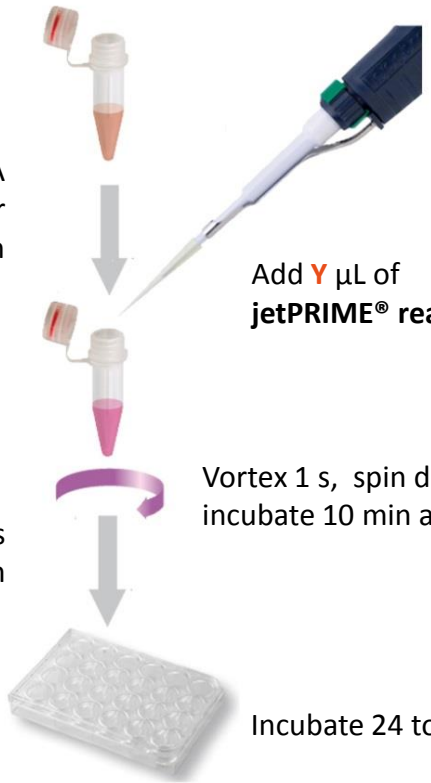
Quantities per well, dish or flask

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

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 (10nM)	X = amount of siRNA added (50nM)	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 http://www.polyplus-transfection.com/resources/product-literature/

Polyplus transfection® THE DELIVERY EXPERTS

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Version H

jetPRIME® transfection reagent
Short protocol – Optimization Tips (siRNA)

Protocol Optimization

- Check our online Cell Transfection Database for cell specific protocols at: http://www.polyplus-transfection.com/resources/cell-transfection-database/
- Test different siRNA concentration ranging from 10 to 50 nM (final concentration).
- Use cells at 50% confluency at time of transfection.

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: housekeeping gene (GAPDH or HPRT) or fluorescently labeled 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, virus production and DNA/siRNA cotransfection, please refer to the complete protocol available online at:
http://www.polyplus-transfection.com/resources/product-literature/

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