jetPRIME® transfection reagent
Short protocol – siRNA Transfection

DAY 0: Cell seeding = 50% confluency at the time of transfection

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

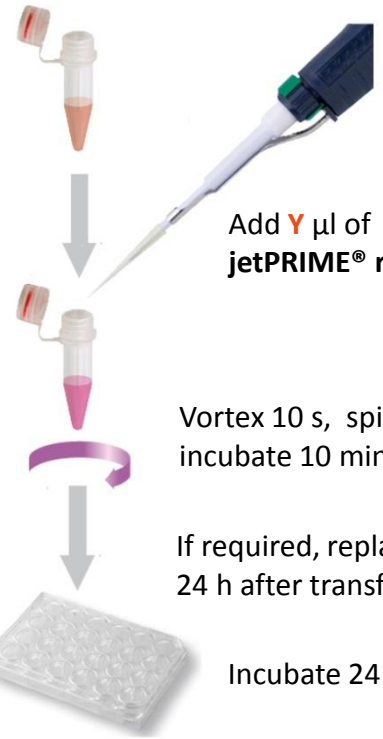
Quantities per well, dish or flask

Culture vessel	Number of cells	V volume of serum containing medium during transfection
24-well	25 000 – 40 000	0.5 ml
12-well	50 000 – 80 000	1 ml
6-well / 35 mm	100 000 – 250 000	2 ml

DAY 1: Transfection

→ Perform transfection **in the presence of serum**
→ Use **jetPRIME® buffer only**
→ Transfect cells at **60-80% 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 10 s, spin down and
incubate 10 min at RT

Add transfection mix
to the cells in serum
containing medium

If required, replace medium
24 h after transfection

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)	5.5 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

DAY 2-3: Measure gene expression

See back page for optimization tips
Download complete protocol on http://www.polyplus-transfection.com/resources/product-literature/

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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 after 4 h
- 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® (4°C) and the siRNA
- 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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