

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

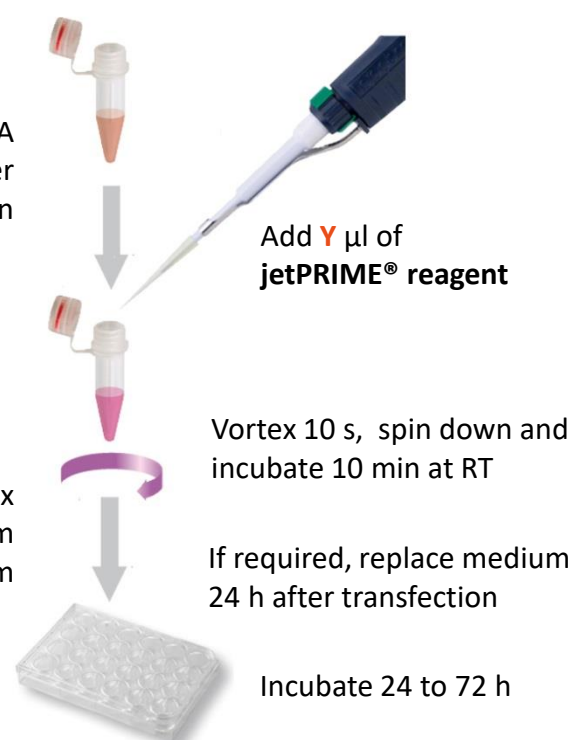
DAY 1: Transfection

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

→ Use **jetPRIME[®] buffer only**

Dilute **X** pmoles of siRNA
in **W** μ l of jetPRIME[®] buffer
Vortex 10 s and spin down

Add transfection mix
to the cells in serum
containing medium



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 μ l	5.5 pmoles (76 ng)	5.5 pmoles (381 ng)	2 μ l
12-well	100 μ l	11 pmoles (152 ng)	55 pmoles (762 ng)	3 μ l
6-well / 35 mm	200 μ l	22 pmoles (306 ng)	110 pmoles (1524 ng)	4 μ l

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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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/>