jetPRIME® Transfection Reagent

Short Protocol – siRNA Transfection

Day 0: Cell Seeding

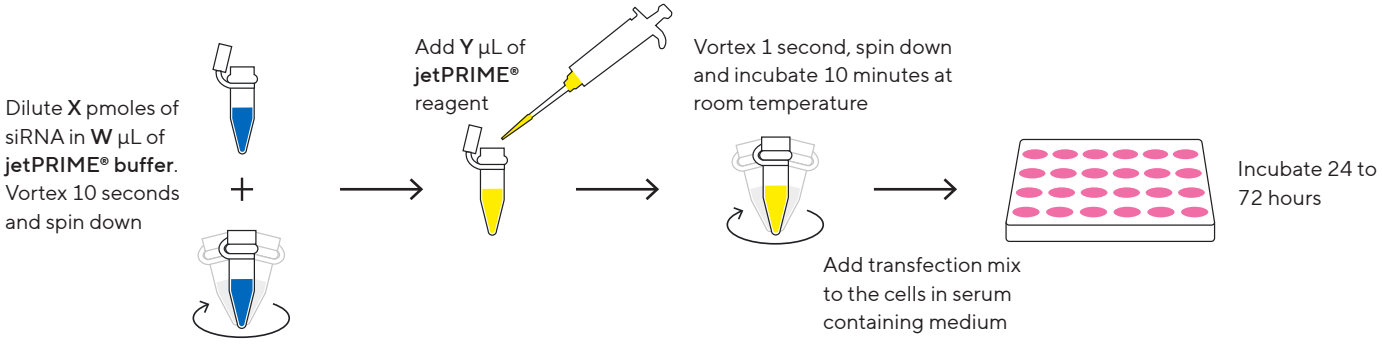
- Seed cells in **V** mL of cell growth medium according to the table below

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 cm²	0.5 x 10^6 – 1 x 10^6	10 mL

Quantities per well, dish or flask.
*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**



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 cm²	500 µL	105 pmoles (1460 ng)	525 pmoles (7274 ng)	20 µL

Quantities per well, dish or flask.

Day 2 – 3: Measure Gene Expression

See back page for optimization tips.

Download complete protocol on sartorius.com

Short Protocol – Optimization Tips (siRNA)

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 website at www.sartorius.com.

Tips to Increase Cell Viability of Sensitive Cells

- Replace medium 4 hours 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 hours 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 on www.sartorius.com.

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