SARTORIUS

Short Protocol

jetOPTIMUS® Transfection Reagent

DNA Transfection

Day 0: Cell Seeding

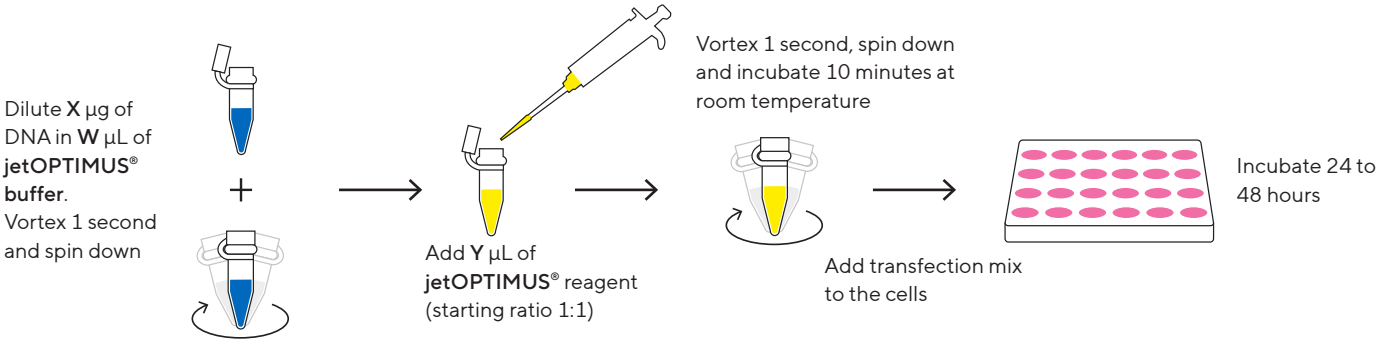
- Seed cells in **V** mL of cell growth medium according to the table below

Culture vessel	Number of cells*	V = volume of medium during transfection [mL]
96-well	7,500 – 25,000	0.125
24-well	40,000 – 100,000	0.5
12-well	80,000 – 200,000	1
6-well/35 mm	150,000 – 400,000	2
60 mm/flask 25 cm^2	200,000 – 850,000	5
100 mm/flask 75 cm^2	1×10^6 – 4×10^6	10

Quantities per well, dish or flask.

Day 1: Transfection Using jetOPTIMUS® Reagent

- Use **jetOPTMUS® buffer only**
- Transfect cells at **60 – 80% confluency**



Culture vessel	W = volume of jetOPTIMUS® buffer [µL]	X = amount of DNA added [µg]	Y = volume of jetOPTIMUS® reagent [µL]
96-well	12.5	0.13	0.13 – 0.19
24-well	50	0.5	0.5 – 0.75
12-well	100	1	1 – 1.5
6-well/35 mm	200	2	2 – 3
60 mm/flask 25 cm^2	500	4	4 – 6
100 mm/flask 75 cm^2	1,000	10	10 – 15

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

Protocol Optimization

- Test different DNA amounts: X, 0.5X and 1.5X
- Test different DNA/jetOPTIMUS® ratios, 1:1 to 1:1.5.
- For cell specific protocols, visit our website at www.sartorius.com.

Culture vessel	W = volume of jetOPTIMUS® buffer [µL]	X = amount of DNA added [µg]	Y = volume of jetOPTIMUS® reagent [µL]
96-well	12.5	0.10 – 0.20	0.10 – 0.30
24-well	50	0.25 – 0.75	0.25 – 1
12-well	100	0.5 – 1.5	0.5 – 2.25
6-well/35 mm	200	1 – 3	1 – 4.5
60 mm/flask 25 cm^2	500	2 – 6	2 – 9
100 mm/flask 75 cm^2	1,000	5 – 15	5 – 22

Quantities per well, dish or flask.

Tips to Increase Cell Viability of Sensitive Cells

- Replace medium 4 hours after transfection.
- Decrease DNA amount to 0.5X while maintaining the DNA/jetOPTIMUS® ratio previously used.
- Analyze transfection at an earlier time point (24 hours after transfection instead of 48 hours for instance).
- Perform transfection in reduced serum medium for sensitive cells.
- Check that the target gene does not affect cell viability.
- Ensure that cells have been passaged more than twice and less than 20 times prior to transfection.
- Discard overconfluent cells.

Good mRNA Transfection Practices

- Store appropriately jetOPTIMUS® (5 ± 3 °C).
- Regularly check for mycoplasma contamination.
- Use a reporter gene to set up and optimize transfection conditions.
- 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:
Please refer to the complete protocol available on www.sartorius.com.

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