jetOPTIMUS® transfection reagent

Short protocol – DNA transfection

DNA TRANSFECTION

Day 0: Cell seeding

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

Quantities per well, dish or flask

Culture vessel	Number of cells*	V = volume of medium during transfection
96-well	7500 - 25 000	0.125 mL
24-well	40 000 - 100 000	0.5 mL
12-well	80 000 - 200 000	1 mL
6-well / 35 mm	150 000 - 400 000	2 mL
60 mm / flask 25 cm^2	200 000 - 850 000	5 mL
100 mm / flask 75 cm^2	1 x 10^6 - 4 x 10^6	10 mL

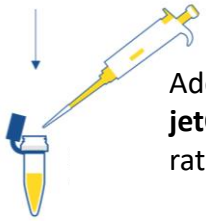
*For specific cell type or suspension cells, please refer to the complete protocol.

Day 1: Transfection using jetOPTIMUS® reagent

→ Use jetOPTMUS® **buffer only**

→ Transfect cells at **60-80% confluency**

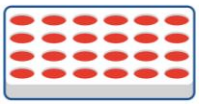
Dilute **X** µg of DNA in **W** µL of **jetOPTIMUS® buffer**. Vortex 1 s and spin down



Add **Y** µL of **jetOPTIMUS®** reagent (starting ratio 1:1)

Vortex 1 s, spin down and incubate 10 min at RT

Add transfection mix to the cells



Incubate 24 to 48 h

Quantities per well, dish or flask

Culture vessel	W = volume of jetOPTIMUS® buffer	X = amount of DNA added	Y = volume of jetOPTIMUS® reagent
96-well	12.5 µL	0.13 µg	0.13 – 0.19 µL
24-well	50 µL	0.5 µg	0.5 – 0.75 µL
12-well	100 µl	1 µg	1 – 1.5 µL
6-well / 35 mm	200 µL	2 µg	2 – 3 µL
60 mm / flask 25 cm^2	500 µL	4 µg	4 – 6 µL
100 mm / flask 75 cm^2	1000 µL	10 µg	10 – 15 µL

Day 2-3: Measure gene expression

See back page for optimization tips

Download complete protocol on https://myaccount.polyplus-transfection.com/

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jetOPTIMUS® transfection reagent

Short protocol – Optimization tips

Protocol Optimization

- Test different DNA amounts: X, 0.5 X and 1.5 X
- Test different DNA/jetOPTIMUS® ratios, 1:1 to 1:1.5.
- For cell specific protocols, check our online Cell Transfection Database: http://www.polyplus-transfection.com/resources/cell-transfection-database/

Quantities per well, dish or flask

Culture vessel	W = volume of jetOPTIMUS® buffer	X = amount of DNA added	Y = volume of jetOPTIMUS® reagent
96-well	12.5 µL	0.10 – 0.20 µg	0.10 – 0.30 µL
24-well	50 µL	0.25 – 0.75 µg	0.25 – 1 µL
12-well	100 µL	0.5 – 1.5 µg	0.5 – 2.25 µL
6-well / 35 mm	200 µL	1 – 3 µg	1 – 4.5 µL
60 mm / flask 25 cm^2	500 µL	2 – 6 µg	2 – 9 µL
100 mm / flask 75 cm^2	1000 µL	5 – 15 µg	5 – 22 µL

Tips to increase cell viability of sensitive cells

- Replace medium 4 h after transfection.
- Decrease DNA amount to 0.5 X while maintaining the DNA/jetOPTIMUS® ratio previously used.
- Analyze transfection at an earlier time point (24 h after transfection instead of 48 h 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 DNA 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 when creating your account online at: https://myaccount.polyplus-transfection.com/.

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