jetOPTIMUS® transfection reagent
Short protocol - DNA Transfection

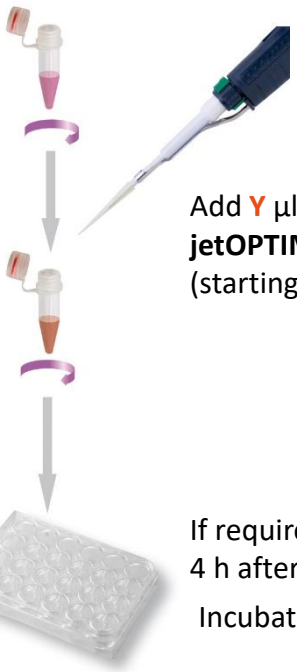
DAY 0: Cell seeding

→ 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
96-well	7500 - 25 000	0.125 mL
24-well	40 000 - 100 000	0.5 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

DAY 1: Transfection using jetOPTIMUS® reagent



Dilute X µg of DNA
in W µl of jetOPTIMUS® buffer
Vortex 1 second and spin down

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

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

Add transfection mix to cells in
serum-containing medium

If required, replace medium
4 h after transfection

Incubate 24 to 48 h

→ Transfect cells at **60-80% confluency**
→ Use **jetOPTIMUS® buffer only**
→ Perform transfection **in the presence of serum**

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
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 www.polyplus-transfection.com/resources/product-literature/

Polyplus transfection® THE DELIVERY EXPERTS

Contact us:
Phone: +33 (0)3 90 40 61 80
Email: support@polyplus-transfection.com
Website: www.polyplus-transfection.com

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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

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
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

- Check our online Transfection Database for cell specific protocols at:
www.polyplus-transfection.com/resources/cell-transfection-database/

Tips to increase cell viability of sensitive cells

- Replace medium after 4 h.
- 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).
- 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 online at:
www.polyplus-transfection.com/resources/product-literature/

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