PROTOCOL

jetOPTIMUS®
in vitro DNA transfection reagent

DESCRIPTION

jetOPTIMUS® is an innovative cationic nanotechnology developed to improve cellular uptake and endosomal escape of DNA in adherent cells resulting in higher transfection efficiency, even in hard-to-transfect cells. In order to work in relevant physiological conditions, transfection with jetOPTIMUS® requires a minimum DNA quantity and reagent volume which preserves cell viability and morphology.

1. DNA transfection protocol (Forward)

1.1. Cell seeding

For optimal transfection of adherent cells using jetOPTIMUS® reagent, cells should be seeded the day before transfection to reach 60 to 80 % confluency at the time of transfection. Typically, for experiments in 24-well plates, ~50 000 adherent cells are seeded per well in 500 µL complete growth medium 24 h prior to transfection. For other formats, please refer to Table 1:

Table 1. Recommended number of cells to seed the day before transfection.

Culture vessel	Number of cells to prepare per well	Surface area per well (cm^2)	Volume of growth medium to seed the cells (mL)
96-well	7500 - 25 000	0.3	0.125
24-well	40 000 - 100 000	1.9	0.5
6-well / 35 mm	150 000 - 400 000	9.4	2
60 mm / flask 25 cm^2	200 000 - 850 000	25 - 28	5
100 mm / flask 75 cm^2	1 x 10^6 - 4 x 10^6	75 - 78.5	10

1.2. DNA Transfection protocol

The following protocol is given for transfection of DNA at 0.5 µg per well in a 24-well plate. For other culture formats, please refer to Table 2.

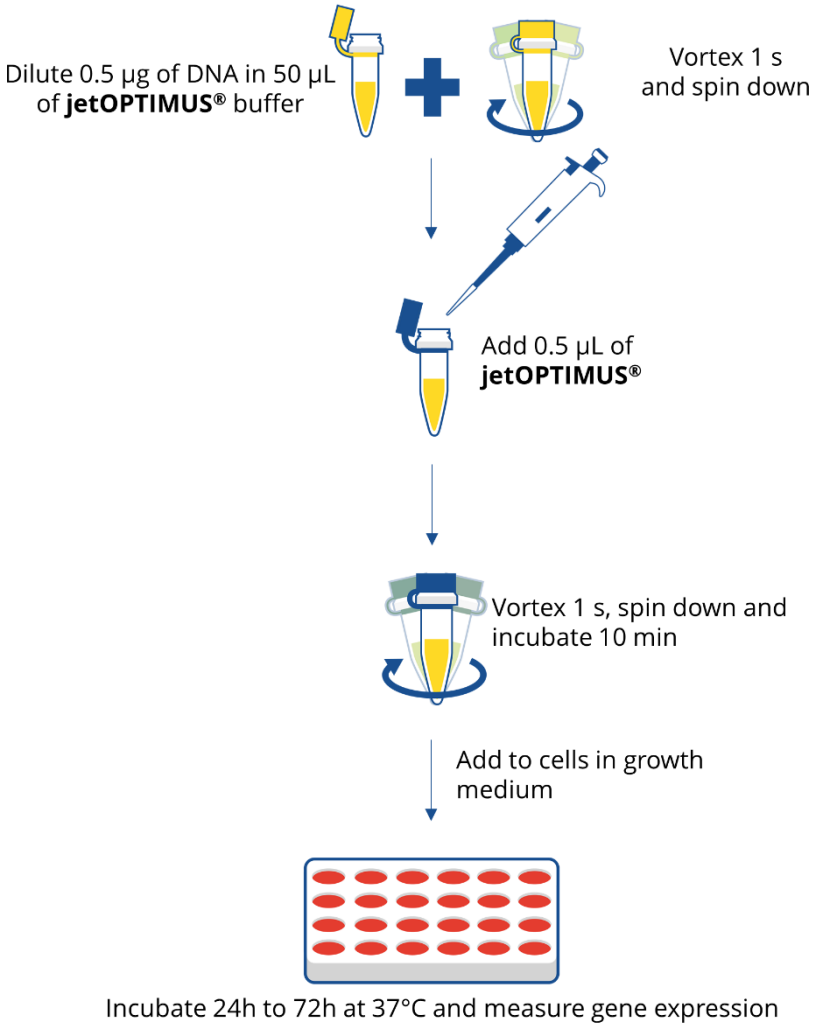
1. Dilute 0.5 µg DNA into 50 µL jetOPTIMUS® buffer. Vortex for 1 second and spin down briefly.
2. Vortex jetOPTIMUS® reagent for 5 seconds and spin down before use.
3. Add 0.5 µL jetOPTIMUS® onto the DNA solution (ratio 1:1 corresponding to μg_{DNA}:$\mu L_{reagent}$), **vortex for 1 second** and spin down briefly.
4. Incubate for 10 minutes at room temperature.
5. Add 50 µL of transfection mix per well dropwise onto the cells in serum containing medium and distribute evenly.
6. Gently rock the plates back and forth and from side to side, incubate the plate at 37 °C.
7. If needed, replace transfection medium after 4 hours by cell growth medium and return the plates to the incubator.
8. Analyze transgene expression after 24 hours or later.

Table 2. DNA transfection guidelines according to the cell culture vessel used.

Culture vessel	Volume of jetOPTIMUS® buffer (µL)	Amount of DNA (µg)	Volume of jetOPTIMUS® reagent (µL)	Volume of growth medium (mL)
96-well	12.5	0.13	0.13 - 0.19	0.125
24-well	50	0.5	0.5 - 0.75	0.5
6-well / 35 mm	200	2	2 - 3	2
60 mm / flask 25 cm^2	500	4	4 - 6	5
100 mm / flask 75 cm^2	1000	10	10 - 15	10

NOTE: jetOPTIMUS® buffer must be used for successful transfection.

jetOPTIMUS® transfection protocol for 24-well plates



2. Reverse DNA transfection protocol

In this procedure, DNA and jetOPTIMUS® reagent are prepared as a master mix and distributed into the wells. The cells are subsequently added into the wells.

2.1. Cell preparation

The day of transfection, split cells and resuspend them in complete growth medium (with or without serum and antibiotics), at the recommended cell density provided in Table 3.

Typically, for experiments in 96-well plates, a cell suspension of 2 x 10^5 adherent cells/mL is prepared in culture medium on the day of transfection. 0.125 mL of cell suspension is added per well to the complexes. For other culture formats, refer to Table 3.

jetOPTIMUS® is compatible with the presence of serum and antibiotics, therefore you may use serum and antibiotic containing medium during the entire experiment.

Table 3. Recommended seeding cell density.

Culture vessel	Number of cells to prepare per well	Cell density (cells/mL)	Volume of cell suspension per well (mL)
96-well	25 000	2 x 10^5	0.125
24-well	100 000		0.5
6-well/35 mm	400 000		2
60 mm / flask 25 cm ²	800 000		5
100 mm / flask 75 mm²	3 x 10^6		10

NOTE: The optimal cell density for transfection should be determined for every new cell type to be transfected and kept constant in future experiments.

2.2. Reverse DNA transfection protocol

The following conditions are given per well in a 96-well plate. For other culture formats and optimization guidelines, please refer to Tables 4 and 5.

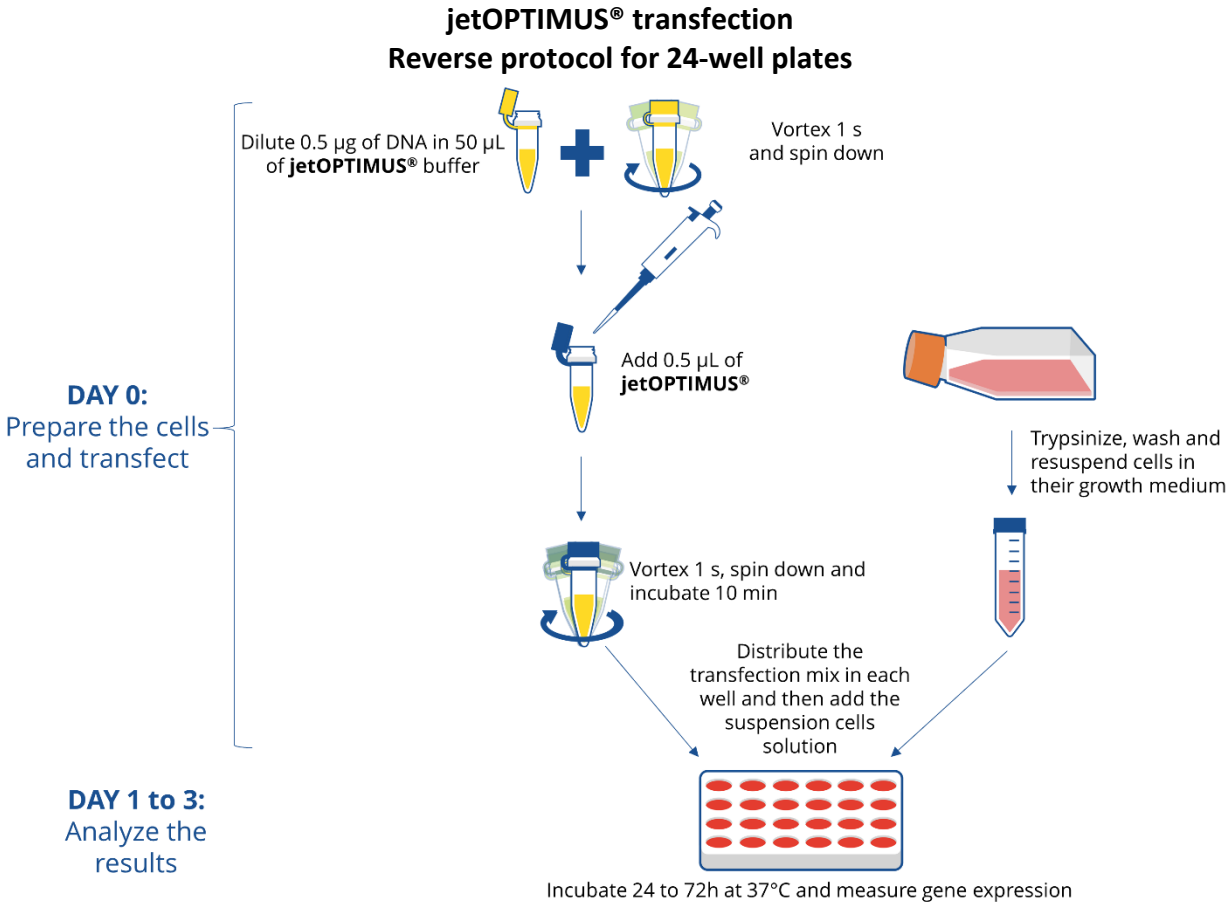
1. On the day of transfection, dilute 0.13 µg DNA into 12.5 µL jetOPTIMUS® buffer (supplied). Vortex for 1 second and spin down briefly.
2. Vortex jetOPTIMUS® reagent for 5 seconds and spin down before use.
3. Add 0.13 µL jetOPTIMUS® onto the DNA solution (ratio 1:1 corresponding to μg_{DNA}:$\mu L_{reagent}$), **vortex for 1 second** and spin down briefly.
4. Incubate for 10 minutes at room temperature.
5. Add 12.5 µL of transfection mix into each well. Do not allow complexes to stay in the well for more than 30 minutes before adding the cell suspension
6. According to table 4, distribute 0.125 mL of cell suspension per well.
7. Return the plates to the cell culture incubator. If cell toxicity is observed, perform a medium change 4h post-transfection.
8. Analyze transgene expression 24 h to 48 h following transfection.

Table 4. DNA reverse transfection guidelines per well according to the cell culture vessel.

Culture vessel	Volume of jetOPTIMUS® buffer (µL)	Amount of DNA (µg)	Volume of jetOPTIMUS® Reagent (µL)	Volume of cell suspension per well (mL)
96-well	12.5	0.13	0.13 – 0.19	0.125
24-well	50	0.5	0.5 – 0.75	0.5
6-well/35 mm	200	2	2 – 3	2
60 mm / flask 25 cm^2	500	4	4 – 6	5
100 mm / flask 75 cm^2	1000	10	10 – 15	10

NOTE: the provided jetOPTIMUS® buffer should be used for successful transfection with jetOPTIMUS®.

Prepare a master mix of minimum 50 µL to allow accurate pipetting and homogenous preparation of the complexes.



3. Optimization guidelines and conditions for specific cell types

You may adjust the volume of reagent and/or the amount of DNA. We recommend testing different ratios of DNA / jetOPTIMUS® reagent from 1:1 to 1:1.5 (μg_{DNA}:$\mu L_{reagent}$) (see Table 6).

Table 6. Optimization guidelines according to the cell culture vessel used.

Culture vessel	Volume of jetOPTIMUS® buffer (µL)	Amount of DNA (µg)	Volume of jetOPTIMUS® reagent (µL)	Volume of growth medium (mL)
96-well	12.5	0.10 - 0.20	0.10 - 0.30	0.125
24-well	50	0.25 - 0.75	0.25 - 1	0.5
6-well / 35 mm	200	1 - 3	1 - 4.5	2
60 mm / flask 25 cm^2	500	2 - 6	2 - 9	5
100 mm / flask 75 cm^2	1000	5 - 15	5 - 22	10

For HEK-293 and HeLa cells, you may decrease the DNA amount to 0.5X and use the 1:1 DNA / jetOPTIMUS® ratio while keeping the same volume of buffer suggested in the protocol.

Specific conditions for many cell types can be found in our cell transfection database, following this link: www.polyplus-transfection.com/resources/cell-transfection-database/.

3.1. Other applications

For co-transfection of multiple plasmids, the total DNA amount per well/plate should not exceed the DNA amount recommended in Table 2. The ratio to use for each plasmid depends on the size of the plasmids, the plasmid constructs and the desired expression level of each plasmid. Please adjust the ratios according to your application, each plasmid representing at least 10 % of the total DNA amount per well/plate.

For other applications, such as stable transfection and plasmid based CRISPR/Cas9 genome editing, please contact our Scientific Support team for specific recommendations at support@polyplus-transfection.com.

4. Troubleshooting

Observations	Actions
Low DNA transfection efficiency	• Ensure that the nucleic acid is diluted in the provided jetOPTIMUS® buffer. • Ensure that cells have been passaged more than twice and less than 20 times prior to transfection. Discard overconfluent cells. • Ensure that the medium is permissive to the transfection. • Use high-quality plasmid preparation, free of proteins. • Optimize the volume of jetOPTIMUS® reagent and the amount of plasmid DNA added per well. Increase the volume of jetOPTIMUS® reagent first; if insufficient, increase the amount of DNA (both according to Table 2). • Serum quality may drastically affect transfection efficiency. When purchasing a new batch of serum or trypsin, check cell viability as well as transfection efficiency. • Use a plasmid containing a common reporter gene such as Luciferase or GFP as a positive control.
Cellular toxicity	• Ensure that the nucleic acid is diluted in the provided jetOPTIMUS® buffer. • Ensure that the plasmid preparation is endotoxin-free. • Perform transfection in reduced serum medium for sensitive cells. • Analyze transfection at an earlier time point (e.g. at 24 h instead of 48 h). • Decrease the volume of jetOPTIMUS® reagent. • Decrease the amount of plasmid DNA added per well. • Verify the toxicity of the expressed protein. If the expressed protein is toxic for the cells, reduce the amount of plasmid DNA.

5. Product Information

5.1. Ordering Information

Part N°	jetOPTIMUS® Reagent	jetOPTIMUS® Buffer
101000051	0.1 mL	10 mL
101000025	0.75 mL	2 x 60 mL
101000006	1.5 mL	4 x 60 mL
201000001	-	60 mL

5.2. Provided buffer

jetOPTIMUS® reagent is provided with an optimized sterile buffer (jetOPTIMUS® buffer). This buffer **must** be used to ensure successful transfection experiments.

5.3. Content

1.5 mL of jetOPTIMUS® transfection reagent is sufficient to perform 3 000 transfections in 24-well plates or 750 transfections in 6-well plates following the standard protocol (DNA:reagent ratio = 1:1).

5.4. Reagent use and Limitations

For research use only. Not for use in humans.

5.5. Quality control

Every batch of jetOPTIMUS® reagent is tested by DNA transfection of HeLa cells with a GFP-expressing plasmid.

Certificates of Analysis are available online in your Customer Area: https://myaccount.polyplus-transfection.com/wp-login.php

5.6. Formulation and Storage

jetOPTIMUS® and its buffer should be stored at 5 ± 3 °C upon arrival to ensure long term stability. jetOPTIMUS®, as guaranteed and indicated on the Certificate of Analysis, is stable at least for 6 months (Part N° 101000051) to at least one year (Part N° 101000025 and 101000006) when stored appropriately.

jetOPTIMUS® is chemically defined and guaranteed free of animal origin products.

Polyplus-transfection® has been awarded ISO 9001 Quality Management System Certification since 2002, which ensures that the company has established reliable and effective processes for manufacturing, quality control, distribution and customer support.

5.7. Trademarks

Polyplus-transfection® and jetOPTIMUS® are registered trademarks of Polyplus-transfection S.A.

How to cite us: “jetOPTIMUS® (Polyplus-transfection S.A, Illkirch, France)”.

6. Contact information

Do you have any technical question regarding your product?

- Website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87

Contact the friendly Scientific Support team which is composed of highly educated scientists, PhDs and Engineers, with extensive hands-on experience in cell culture and transfection. The Scientific Support is dedicated to help our customers reach their goals by proposing different services such as: protocol optimization, personalized transfection conditions, tailored protocols, etc.

For any administrative question, feel free to contact our administration sales team:

- Reception Phone: +33 3 90 40 61 80
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Please note that the Polyplus-transfection® support is available by phone from 9:00 am to 5:00 pm CEST.