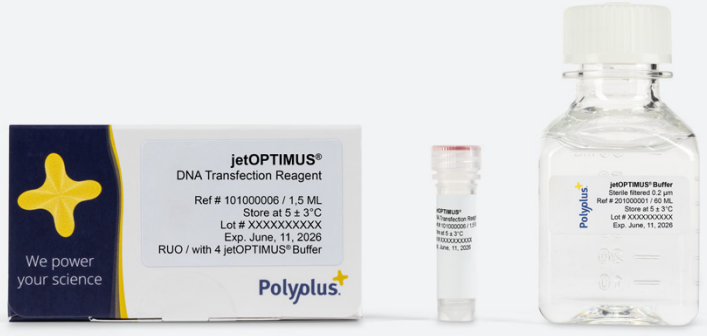
SARTORIUS

Protocol

jetOPTIMUS®

In Vitro DNA Transfection Reagent iPSC Protocol



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.

This protocol is dedicated to the transfection of Induced Pluripotent Stem Cells (iPSCs), always considered as difficult to transfect cells. Due to its specificity of cell culture and its cell sensitivity, transfection conditions need to be adapted to maintain cell viability while keeping a good transfection efficiency.

1 Transfection Protocol

1.1 Cell Seeding

1. The day before transfection, prepare a 1:100 dilution of Matrigel® matrix (Corning®) in cold Gibco™ (Thermo Fisher Scientific) DMEM/F12 Medium and add 250 µL of diluted Matrigel® matrix to each well of a 24-well plate and incubate at 37 °C for 1 hour or more before use.

⚠ NOTE:
Matrigel® matrix-coated plates can be prepared ahead of time and stored for up to 1 week at 4 °C. Equilibrate at 37 °C for 1 hour before plating cells.

2. When iPSC cultures are at 75 – 80% confluent, remove mTeSR1 medium and gently wash the cells with 2 mL of DPBS (without calcium and magnesium) per well in a 6-well plate.
3. Add 1 mL of Accutase™ (STEMCELL™) or TrypLE™ Select (Thermo Fisher Scientific) solution per well and incubate at 37 °C for 3 – 10 minutes.
4. Observe cultures under a microscope. When individual cells inside colonies have contracted add 1 mL of mTeSR™ medium (STEMCELL™) and detach cells with a cell scraper or by washing the well 3 times.

⚠ IMPORTANT:
Maintain small clumps of 3 – 5 cells to promote efficient transfection, viability, and pluripotency.

5. Centrifuge the cell in a 15 mL conical tube at 200 g during 5 minutes at room temperature.
6. Remove supernatant and suspend cells in 1 mL of mTeSR™ 1 complete medium with 10 µM ROCK Inhibitor.
7. Count cells and make a suspension at 200,000 cells per well in mTeSR™ 1 complete medium with 10 µM ROCK Inhibitor.
8. Add 0.5 mL of the iPSC suspension per well to plate 100,000 cells in precoated 24-well plate.
9. Return the plate to the incubator and culture at 37 °C with 5% CO_2 overnight.

⚠ IMPORTANT:
Plating iPSCs more than 1 day before transfection can lead to a too large colony and lowered the transfection efficiency.

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	20,000	0.32	0.125
24-well	100,000	1.9	0.5
6-well/35 mm	400,000	9.6	2
60 mm/flask 25 cm^2	1,000,000 – 1,500,000	25	5
100 mm/flask 75 cm^2	3,000,000 – 4,000,000	75	10

Notes: The optimal cell density for transfection should be determined for every new cell type to be transfected and kept constant in future experiments. jetOPTIMUS® is also compatible with other matrices such as Vitronectin (VTN-N) Recombinant Human Protein (Thermo Fisher Scientific) or Geltrex™ (Gibco™).

1.2 Transfection Protocol

The following conditions are given per well in a 24-well plate. For other culture formats and optimization guidelines, please refer to Table 2.

1. On the day of transfection, remove the culture medium and add 0.5 mL of mTeSR™ 1 complete medium at least 30 minutes before transfection and return the cells at to the incubator at 37 °C + 5% CO_2.

⚠ NOTE:
Addition of ROCK Inhibitor on the day of transfection is not necessary but can promote cells survival during transfection.

2. Dilute 0.5 µg DNA into 50 µL jetOPTIMUS® buffer (supplied). Vortex for 1 second and spin down briefly.
3. Vortex jetOPTIMUS® reagent for 5 seconds and spin down before use.
4. Add 0.75 µL jetOPTIMUS® onto the DNA solution (ratio 1:1.5 corresponding to μg_{DNA}:$\mu L_{reagent}$), vortex **for 1 second** and spin down briefly.
5. Incubate for 10 minutes at room temperature.
6. Add 50 µL of transfection mix onto the cells.
7. Return the plates to the cell culture incubator. If cell toxicity is observed, add 0.5 mL of mTeSR™ 1 complete medium 4 hours post-transfection.
8. Perform reporter gene assay 24 to 48 hours following transfection.

jetOPTIMUS® iPSC Transfection Protocol for 24-Well Plates

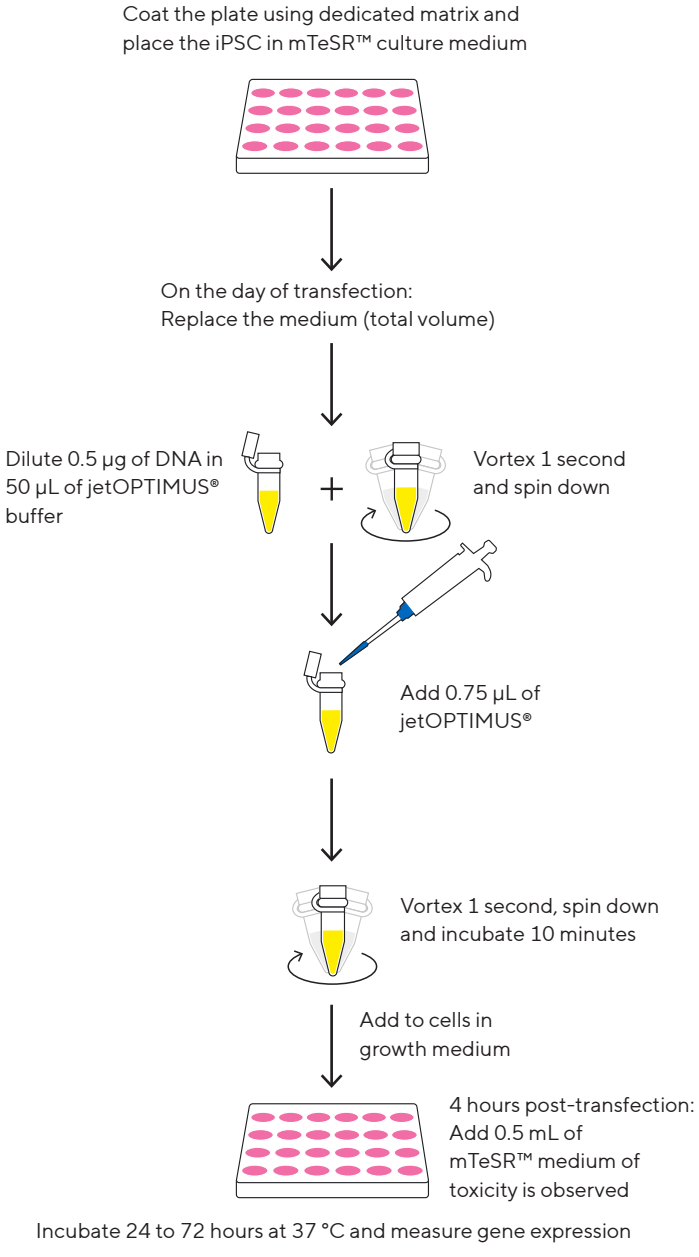


Table 2: *DNA Transfection Guidelines According to the Cell Culture Vessel Used*

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	0.2	0.3	0.125
24-well	50	0.5	0.75	0.5
6-well/35 mm	200	2	3	2
60 mm/flask 25 cm^2	500	5	7.5	5
100 mm/flask 75 cm^2	1,000	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.

1.3 Optimization Guidelines and Conditions for Specific Cell Types

Table 3: *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	0.2	0.2 - 0.4	0.125
24-well	50	0.5	0.5 - 1	0.5
6-well/35 mm	200	2	2 - 4	2
60 mm/flask 25 cm²	500	5	5 - 10	5
100 mm/flask 75 cm²	1,000	10	10 - 20	10

2 Troubleshooting

Observations	Actions
High Toxicity	Reduce DNA amount or add ROCK Inhibitor when the medium is diluted.
Only borders of colonies are transfected	Ensure that the cells aren't forming too compact colonies by reducing the clumps size.
Low | No efficiency	• Ensure that the medium/ matrix is compatible with transfection. • Ensure that the promoter isn't silenced in the cell line; some promoters such as the cytomegalovirus (CMV) promoter can be transcriptionally silenced in iPSCs.
Too much differentiated cells in well	Remove all the differentiated parts before seeding the cells.

3 Product Information

3.1 Ordering Information

Part number	jetOPTIMUS® reagent vial size [mL]	jetOPTIMUS® buffer [mL]
101000051	0.1	10
101000025	0.75	2x60
101000006	1.5	4x60
201000001	-	60

3.2 Content

1.5 mL of jetOPTIMUS® transfection reagent is sufficient to perform 2,000 transfections in 24-well plates or 500 transfections in 6-well plates following the standard protocol.

3.3 Reagent Use and Limitations

For research use only. Not for use in humans.

3.4 Quality Control

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

3.5 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.

3.6 Trademarks

Polyplus-transfection® and jetOPTIMUS® are registered trademarks of POLYPLUS-TRANSFECTION S.A.
How to cite us: "jetOPTIMUS® (Polyplus-transfection S.A, Illkirch, France)".

Germany
Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further information, visit
sartorius.com

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