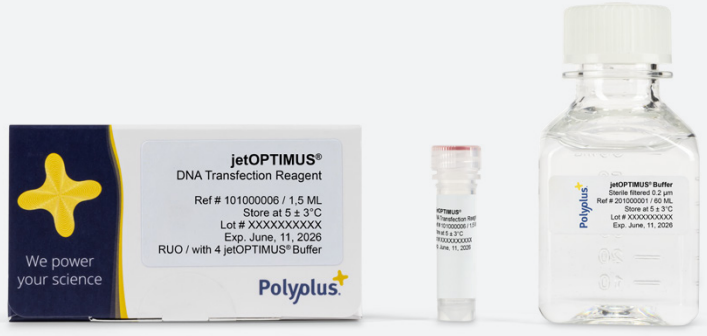
SARTORIUS

Protocol

jetOPTIMUS®

In Vitro DNA Transfection Reagent Stem Cells



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 primary stem cells, 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

Depending on the cell type, seed cells in complete growth medium (with or without serum and antibiotics). Typically, the cell density needs to be between 50 and 70% of confluence the day of the transfection.

Table 1: *Recommended Number of mES 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	5,000	0.32	0.125
24-well	20,000	1.9	0.5
6-well/35 mm	80,000	9.6	2
60 mm/flask 25 cm^2	400,000 – 600,000	25	5
100 mm/flask 75 cm^2	1,000,000 – 2,000,000	75	10

Table 2: *Recommended Number of hMS Cells to Seed Three Days Before Transfection*

Culture vessel	Number cells to prepare per well	Surface area per well [cm^2]	Volume of growth medium to seed the cells [mL]
96-well	3,000	0.32	0.125
24-well	12,000	1.9	0.5
6-well/35 mm	48,000	9.6	2
60 mm/flask 25 cm^2	120,000	25	5
100 mm/flask 75 cm^2	500,000	75	10

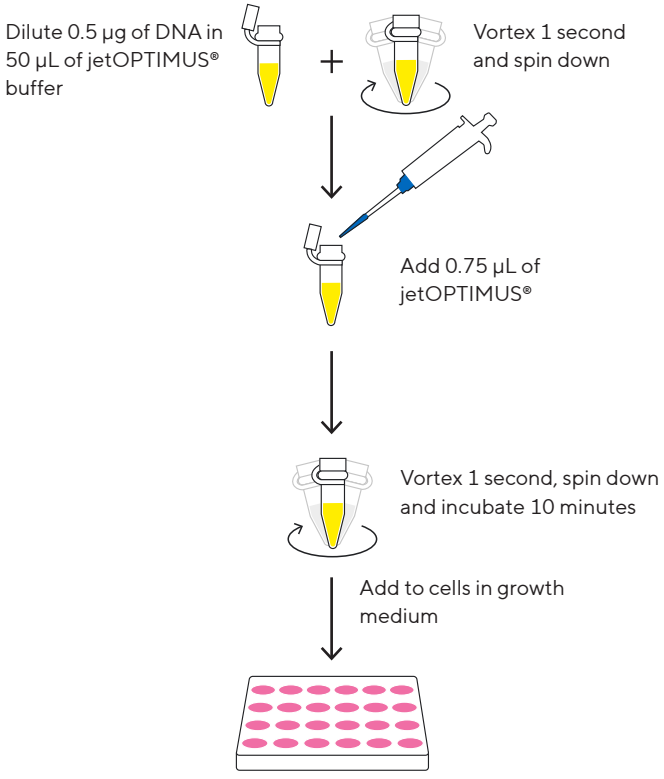
Note: The optimal cell density for transfection should be determined for every new cell type to be transfected and kept constant in future experiments.

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 3 and 4.

1. On the day of transfection, dilute 0.5 µg DNA into 50 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 0.75 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.
4. Incubate for 10 minutes at room temperature.
5. Add 50 µL of transfection mix onto the cells.
6. Return the plates to the cell culture incubator. If cell toxicity is observed, perform a medium change 4 hours post-transfection.
7. Perform reporter gene assay 24 to 48 hours following transfection.

jetOPTIMUS® Stem Cell Transfection Protocol for 24-Well Plates



Incubate 24 to 72 hours at 37 °C and measure gene expression

Table 3: *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 4: *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	0.4 – 0.6	0.4 – 1.2	0.5
6-well/35 mm	200	2	2 – 3	2
60 mm/flask 25 cm^2	500	5	5 – 7.5	5
100 mm/flask 75 cm^2	1,000	10	10 – 15	10

2 Troubleshooting

Observations	Actions
Low efficiency	• Ensure that culture medium doesn't inhibit transfection or replace it by transfection medium during 4 hours. • Some cell lines lost their transfection permeability after too many passages. Ensure to use cells at early passage. • Ensure that the nucleic acid is diluted in the provided jetOPTIMUS® buffer. • Use a plasmid containing a common reporter gene such as Luciferase of GFP as positive control.
High toxicity	• Lower DNA quantity and	or jetOPTIMUS® volume per well. • Replace or dilute medium 4 hours post-transfection. • Analyze transfection at an earlier time point (e.g. at 24 hours instead of 48 hours). • Verify the toxicity of the expressed protein. If the expressed protein is toxic for the cells, reduce the amount of plasmid DNA.

3 Product Information

3.1 Ordering Information

Part number	jetOPTIMUS® reagent vial size	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

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 (DNA:reagent ratio = 1:1.5).

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 Number 101000051) to at least one year (Part Numbers 101000025 and 101000006) when stored appropriately.

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

3.6 Trademarks

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