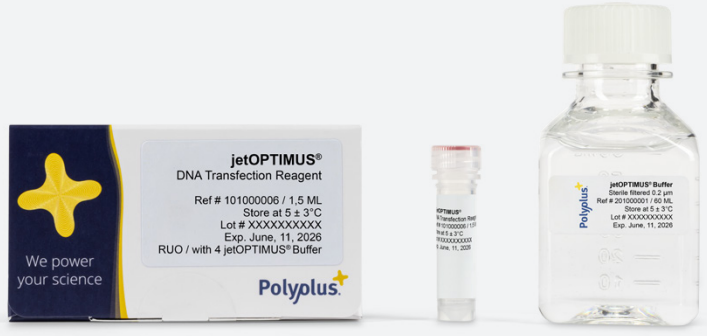
SARTORIUS

Protocol

jetOPTIMUS®

In Vitro DNA Transfection Reagent Neuron 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 primary neurons, 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 Pre-coating Material

The development and survival of neurons requires biological coating or chemical modification of the plate surface. The coating nature may vary depending on the specific neuronal type.

For example, Poly-D-lysine and Poly-L-Lysine are widely used for cortex and hippocampal primary neuron culture.

Table 1: *Coating Solutions*

Cells	Coating solution	Reference	Quantities
Cortex and hippocampal neurons	Poly-D-lysine	P7886, SIGMA	4,5 µg/cm²
Striatum neurons and Dorsal Root Ganglion	Poly-L-lysine	0413, SCIENCELL	2 µg/cm²
DopaNeurons (induced pluripotent stem cell-derived)	Poly-L-Ornithin and laminin	P4957 and L2020, SIGMA	32 µg PLO/cm² and 2 µg laminin/cm²

1.2 Cell Seeding

Cell seeding densities and culture conditions depend on neuron type and source (fresh or frozen-thawed cells). As cell mortality is often observed upon cells thawing, we recommend to increase the seeding density of thawed cells compared to fresh neurons.

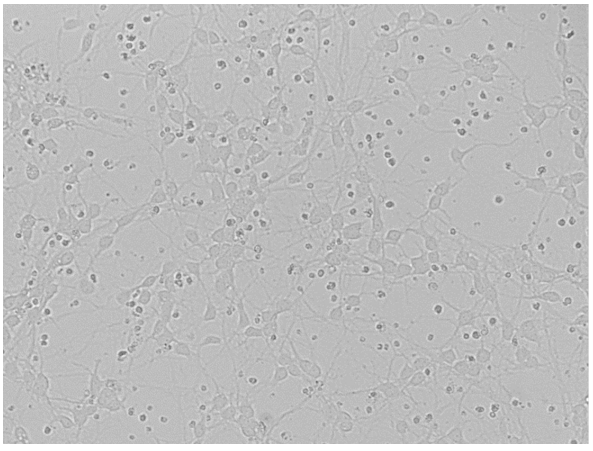
For cell density guidelines for fresh | thawed cortex or hippocampal neuron culture, please refer to Table 2 and 3. The viable cell number may need to be further optimized according to the specific culture conditions.
Inhibitors, as the ARA-C (Arabinosylcytosine), for example, can be used to reduce glial cell proliferation in the fresh culture.

Important: to limit fresh neurons mortality, ensure that no further manipulation of the culture occurs for at least 24 hours after seeding. For thawed neurons, refer to supplier recommendations. Do not expose neurons to air at any time.

Table 2: *Recommended Number of Viable Cells to Seed for Thawed Neurons*

Culture vessel	Number of viable cells to prepare per well	Surface area per well [cm²]	Volume of growth medium to seed the cells [mL]
48-well	75,000 – 100,000	0.95	0.5
24-well	150,000 – 200,000	1.9	1
6-well/35 mm	750,000 – 1,000,000	9.4	3

Figure 1: *Example of Rat Cortical Neuron Culture*

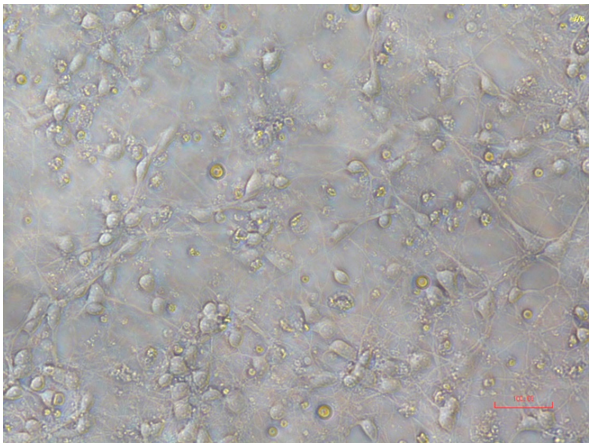


Note. This image shows thawed cortical neurons at day 4 (thawing density = 100,000 viable cells/cm²) into Poly-D-Lysine-coated 48-well plate.

Table 3: *Recommended Number of Viable Cells to Seed for Fresh Neurons.*

Culture vessel	Number of viable cells to prepare per well	Surface area per well [cm²]	Volume of growth medium to seed the cells [mL]
48-well	25,000 – 37,500	0.95	0.5
24-well	50,000 – 75,000	1.9	1
6-well/35 mm	250,000 – 375,000	9.4	3

Figure 2: *Example of Fresh Mouse Cortical Neuron Culture*



Note. This image shows fresh cortical neurons at day 5 after seeding (seeding density = 50,000 viable cells/cm²) into Poly-D-Lysine-coated 24-well plate.

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

1.3 Transfection Protocol

Neurons must be transfected when the neurite network is correctly developed. This typically occurs 4 to 5 days after seeding for thawed cells, and 5 to 7 days for fresh neurons. We recommend assessing neurons' development through direct microscopic observations.

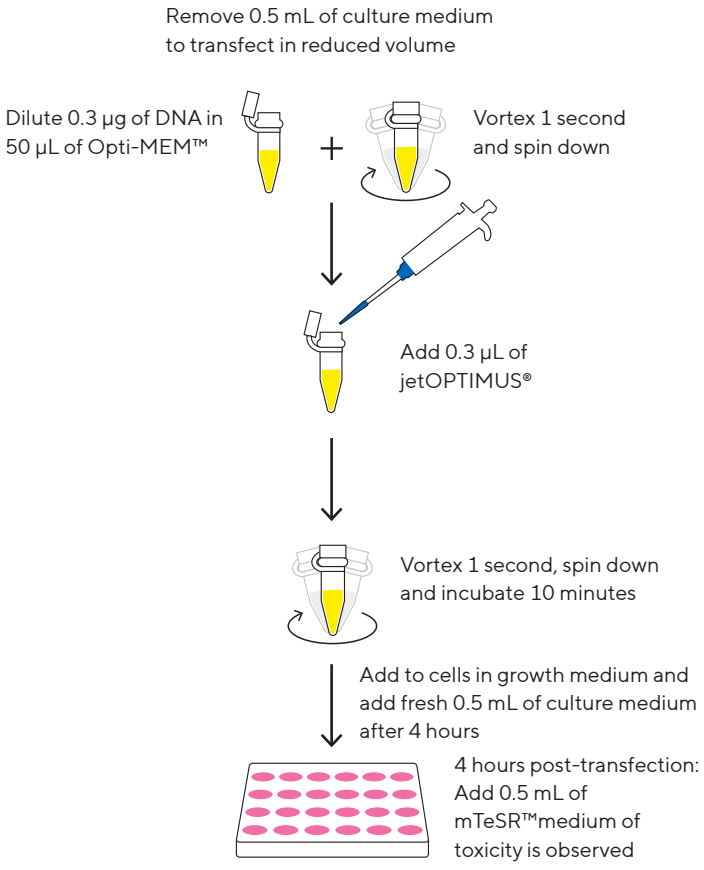
Table 4: *DNA Transfection Guidelines According to the Cell Culture Vessel Used*

Fresh and thawed neurons				
Culture vessel	**Volume of DNA buffer [µL]**	**Amount of DNA [µg]**	**Ratio µg DNA:µL jetOPTIMUS®**	**Volume of growth medium per well [mL]**
48-well	25	0.10 – 0.20	1:1	0.25
24-well	50	0.20 – 0.40	1:1	0.5
6-well/35 mm	200	1 – 2	1:1	2

The following protocol is given for transfection of DNA at 0.3 µg per well in a 24-well plate. For other culture formats, please refer to Table 4.

1. Before transfection, remove 0.5 mL (corresponding to half of the culture medium), to transfect in reduced volume.
2. Equilibrate jetOPTIMUS® and Opti-MEM™ at room temperature for optimal complexes preparation.
3. Dilute 0.3 µg DNA into 50 µL Opti-MEM™. Vortex for 1 second and spin down briefly.
4. Vortex jetOPTIMUS® reagent for 5 seconds and spin down before use.
5. Add 0.3 µL jetOPTIMUS® onto DNA solution (ratio 1:1 corresponding to μg_{DNA}:$\mu L_{reagent}$), vortex for 1 second and spin down briefly.
6. Incubate for 10 minutes at room temperature.
7. Add 50 µL of transfection mix per well dropwise onto the cells and distribute evenly.
8. Gently rock the plates back and forth and from side to side, incubate the plate at 37 °C.
9. Add 0.5 mL of pre-warmed complete culture medium after 4 hours of transfection.
10. Analyze gene expression after 24 hours or later.

Neuron Transfection Protocol for 24-Well Plate



1.4 Optimization Guidelines and Recommendations

Before transfection, it is recommended to ensure DNA optimal characteristics (size, type, quality, purity).
Please refer to Table 5 for additional information concerning DNA to use for transfection of neurons with jetOPTIMUS®.

Table 5: *Optimization Guidelines and Recommendations*

Actions
Ensure that the nucleic acid is diluted in Opti-MEM™.
Use high-quality plasmid preparation, free of proteins. Measure the OD 260/280 nm ratio to verify DNA purity
Ensure that the plasmid DNA promoter is efficient and not silenced in neurons. CMV, CAGG and EF1-α can be alternatives.
The plasmid size can be not suitable to transfect neurons. DNA of 3 kb to 6.5 kb enables efficient transfection. jetOPTIMUS® is less efficient for DNA size bigger than 10 kb

2 Troubleshooting

Different experimental parameters may play a role in reducing transfection efficiency and | or cell viability. Please refer to Table 6 for guidance to improve jetOPTIMUS® transfection results. Please keep in mind that transfection conditions must be optimized according to neuron type.

Observations		Actions
Low efficiency	Good cell viability	▪ Ensure that the nucleic acid is diluted in Opti-MEM™. ▪ Ensure that the medium is permissive to the transfection. ▪ Ensure that the DNA amount and DNA:reagent ratio is appropriate for the used cell density. Refer to the optimization ranges provided in table 2, 3 and 4. ▪ Analyze transfection at a later time point (e.g. at 48 hours instead of 24 hours).
Low efficiency	Cellular toxicity	▪ Ensure that the nucleic acid is diluted in Opti-MEM™. ▪ Ensure that the neurite network is developed enough on the transfection day. If needed, transfection may be postponed of one or more days (as required) until full development is reached. ▪ DNA amount and DNA:reagent ratio is too high for the used viable cell density. Refer to the optimization ranges provided in table 2, 3 and 4. ▪ Verify the toxicity of the expressed protein. If the expressed protein is toxic for the cells, reduce the amount of DNA without changing the ratio DNA:jetOPTIMUS® of 1:1. ▪ A part of culture medium can be replaced one day before transfection, without new addition after transfection. The transfection must be carried out in the total culture medium volume, not in reduced volume.
High efficiency	Cellular toxicity	▪ Decrease the amount of plasmid DNA added per well without changing the ratio DNA:jetOPTIMUS® of 1:1. ▪ Analyze transfection at an earlier time point (e.g. at 24 hours instead of 48 hours).

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	2x60 mL
101000006	1.5 mL	4x60 mL
201000001	–	60 mL

3.2 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).

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)".

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