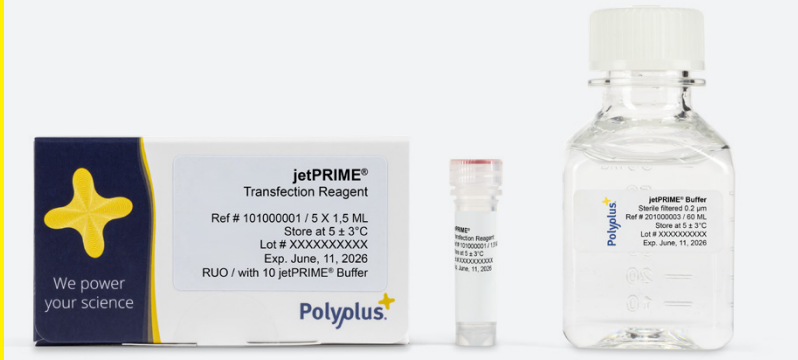
SARTORIUS

Protocol

jetPRIME®

In Vitro DNA and siRNA Transfection Reagent



Description

jetPRIME® is a powerful transfection reagent based on a polymer formulation, ensuring effective and reproducible DNA and siRNA transfection into mammalian cells. jetPRIME® is **extremely efficient** on a wide variety of cell lines. This powerful reagent only requires low amounts of nucleic acid per transfection, hence resulting in **very low cytotoxicity**.

1 Transient DNA Transfection Protocol

1.1 Cell Seeding

For optimal DNA transfection conditions, we recommend using cells which are 60 to 80% confluent at the time of transfection. Typically, for experiments in 6-well plates, ~200,000 cells are seeded per well in 2 mL of cell growth medium 24 hours prior to transfection. For other culture formats, refer to Table 1.

Table 1: *Recommended Number of Cells to Seed the Day Before Transfection*

Culture vessel	Number of cells to seed	Surface area per well [cm^2]	Volume of medium per well to seed the cells [mL]
96-well	7,500 – 25,000	0.3	0.125
24-well	40,000 – 100,000	1.9	0.5
12-well	80,000 – 150,000	3.8	1
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	1×10^6 – 4×10^6	75 – 78.5	10
150 mm/flask 175 cm^2	5×10^6	153 – 175	20

1.2 DNA Transfection Protocol

The following conditions are given per well of a 6-well plate. For other culture format, please refer to Table 2.

1. Dilute 2 µg DNA into 200 µL jetPRIME® buffer (supplied). Mix by vortexing.
2. Vortex jetPRIME® reagent for 5 seconds and spin down before use.
3. Add 4 µL jetPRIME®, vortex for 1 second, spin down briefly.
4. Incubate for 10 minutes at room temperature.
5. Add 200 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 by cell growth medium between 4 to 24 hours after transfection and analyze as required.
8. Analyze after 24 hours or later.

DNA Transfection in 6-Well Plates

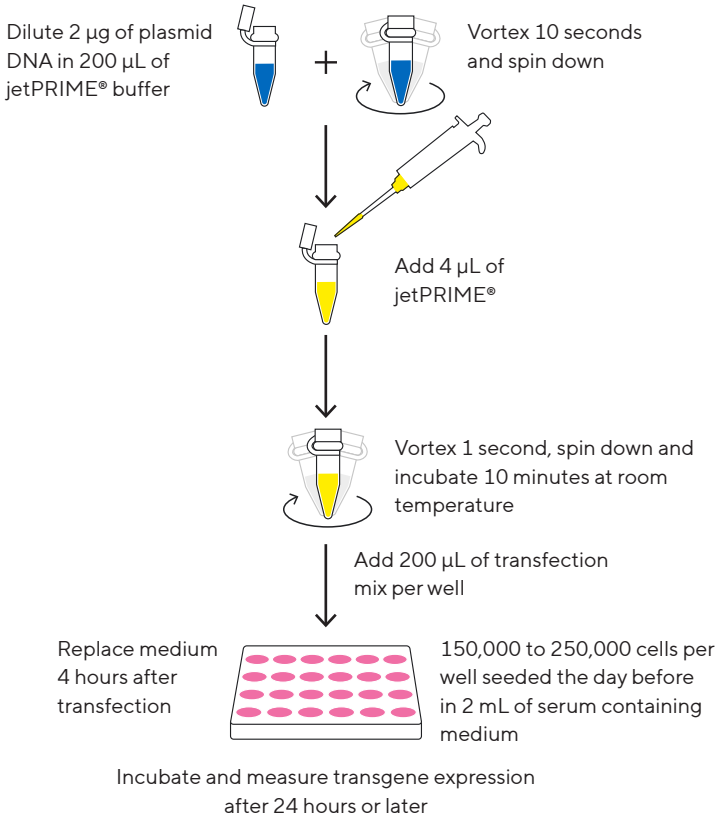


Table 2: *DNA Transfection Guidelines According to the Cell Culture Vessel Used per Well*

Culture vessel	Volume of buffer [µL]	Amount of DNA [µg]	Volume of jetPRIME® reagent [µL]	Volume of growth medium [mL]
96-well*	10	0.1	0.2 - 0.3	0.1
24-well	50	0.5	1 - 1.5	0.5
12-well	75	0.8	1.6 - 2.4	1
6-well/35 mm	200	2	4 - 6	2
60 mm/flask 25 cm²	200	4	8 - 12	5
100 mm/flask 75 cm²	500	10	20 - 30	10
150 mm/flask 175 cm²	1,000	20	40 - 60	20

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

Standard conditions

1:2 DNA to jetPRIME® ratio (w/v)

for 1 µg of DNA use 2 µL of jetPRIME®

⚠ **NOTE:**
jetPRIME® buffer must be used for successful transfection.

1.3 Virus Production in Adherent Cells

jetPRIME® is ideal for virus production, especially retrovirus, AAV and lentivirus, in adherent cells (ex: HEK-293T). For co-transfection of multiple plasmids, the total DNA amount per well/plate should not exceed the DNA amount indicated 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 should represent at least 10% of the total DNA amount per well/plate. The following conditions are given per 100 mm dish. For other culture format, please refer to Table 2. For optimization, please refer to Table 3.

1. Dilute 10 µg total DNA amount into 500 µL jetPRIME® buffer (supplied). Mix by vortexing.
2. Vortex jetPRIME® reagent for 5 seconds and spin down before use.
3. Add 20 µL jetPRIME®, vortex for 1 second, spin down briefly.
4. Incubate for 10 minutes at room temperature.
5. Add 500 µL of transfection mix per dish dropwise onto the cells in serum containing medium and distribute evenly.
6. Gently rock the dish back and forth and from side to side, incubate the dish at 37 °C.
7. Eventually replace transfection medium after 4 hours by cell growth medium and return the dish to the incubator.
8. Incubate 24 to 72 hours and proceed to virus purification and titration.

1.4 Optimization Guidelines and Conditions for Specific Cell Types

Transfection conditions should be optimized for each tested cell line according to the conditions detailed below (Table 3). Visit our website at www.sartorius.com for optimized conditions for various cell lines.

You may adjust the volume of reagent and/or the amount of DNA. The volume of jetPRIME® may range between **2 to 3 µL per µg of DNA** depending on the transfected cell line (**1:2 and 1:3 DNA to jetPRIME®** ratio (w/v)). The amount of DNA may range between 0.5X and 1.5X, X being the amount in µg indicated in Table 2.

Due to the high performance of jetPRIME® reagent (onto HeLa and HEK-293 cells for example), you may decrease the amount of plasmid DNA down to 0.5X (Table 3).

Table 3: *Optimization Guidelines According to the Cell Culture Vessel Used*

Culture vessel	Volume of buffer [µL]	Amount of DNA [µg]	Volume of jetPRIME® reagent [µL]
96-well*	10	0.05 – 0.2	0.1 – 0.6
24-well	50	0.25 – 0.75	0.5 – 2.25
12-well	75	0.4 – 1.2	0.8 – 3.6
6-well/35 mm	200	1 – 3	2 – 9
60 mm/flask 25 cm²	200	2 – 6	4 – 18
100 mm/flask 75 cm²	500	5 – 15	10 – 45
150 mm/flask 175 cm²	1,000	10 – 30	20 – 90

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

2 siRNA Transfection Protocol

2.1 Cell Seeding

For optimal siRNA transfection conditions, we recommend using cells which are 50% confluent at the time of transfection. Typically, for experiments in 6-well plates, 100,000 to 150,000 cells are seeded per well in 2 mL of growth medium 24 hours prior to transfection. For other culture formats, refer to Table 4.

Table 4: *Recommended Number of Cells to Seed the Day Before Transfection*

Culture vessel	Number of adherent cells to seed	Surface area per well [cm^2]	Volume of medium per well to seed the cells [mL]
24-well	24,000 – 40,000	1.9	0.5
12-well	50,000 – 80,000	3.8	1
6-well/35 mm	100,000 – 150,000	9.4	2
60 mm/flask 25 cm^2	200,000 – 500,000	25 – 28	4
100 mm/flask 75 cm^2	0.5×10^6 – 1×10^6	75 – 78.5	10

2.2 siRNA Transfection Protocol

For optimal siRNA-mediated silencing, we recommend using 10 – 50 nM siRNA.

The following conditions are given per well in a 6-well plate. For other culture format, please refer to Table 5.

1. Dilute 22 to 110 pmoles siRNA (for a final concentration of 10 to 50 nM per well) into 200 µL of jetPRIME® buffer. Mix by pipetting up and down.
2. Vortex jetPRIME® reagent for 5 seconds and spin down before use.
3. Add 4 µL jetPRIME® reagent, vortex for 1 second, spin down briefly.
4. Incubate for 10 to 15 minutes at room temperature.
5. Add the transfection mix to the cells in serum containing medium dropwise.
6. Gently rock the dish back and forth and from side to side, incubate the plate at 37 °C.
7. If needed, replace transfection medium by cell growth medium between 4 to 24 hours after transfection and analyze as required.

Table 5: *siRNA Transfection Guidelines According to the Cell Culture Vessel*

Culture vessel	Amount of siRNA [pmoles] 10 nM	Amount of siRNA [pmoles] 50 nM	Volume of jetPRIME® reagent [µL]	Volume of jetPRIME® buffer for complex formation [µL]	Volume of growth medium [mL]	Final volume in the well [mL]
24-well	5.5	27.5	2	50	0.5	0.55
12-well	11	55	3	100	1	1.1
6-well/35 mm	22	110	4	200	2	2.2
60 mm/flask 25 cm^2	42	210	8	200	4	4.2
100 mm/flask 75 cm^2	105	525	20	500	10	10.5

3 DNA and siRNA Cotransfection Protocol

3.1 Cell Seeding

For optimal co-transfection conditions, we recommend using cells which are 60 to 80% confluent at the time of transfection. Typically, for experiments in 6-well plates, 150,000 to 250,000 cells are seeded per well 24 hours prior to transfection. For other culture formats, refer to Table 1.

3.2 DNA and siRNA Cotransfection Protocol

For DNA/siRNA co-transfection experiments, we recommend using 2 µg DNA and 10 to 50 nM siRNA per well in a 6-well plate.

The following conditions are given per well of a 6-well plate. For other culture formats, please refer to Table 6.

1. Dilute 2 µg of DNA and 22 to 110 pmoles siRNA (final concentration: 10 to 50 nM) into 200 µL of jetPRIME® buffer. Mix by pipetting up and down.
2. Vortex jetPRIME® reagent for 5 seconds and spin down before use.
3. Add 4 µL jetPRIME® reagent, vortex for 1 second, spin down briefly.
4. Incubate for 10 to 15 minutes at room temperature.
5. Add the transfection mix to the cells still in serum containing medium dropwise.
6. Gently rock the dish back and forth and from side to side, incubate the plate at 37 °C.
7. If needed, replace transfection medium by cell growth medium 24 hours after transfection and analyze as required.

⚠ NOTE:
If you aim to silence the transfected plasmid with the transfected siRNA, then reduce the amount of DNA to 25% of the quantity indicated in Table 6 (for example: reduce to 500 ng DNA per well of a 6-well plate instead of 2 µg DNA)

The amount of plasmid DNA and the volume of jetPRIME® may be optimized according to Table 3.

Table 6: *DNA and siRNA Co-Transfection Guidelines According to the Cell Culture Vessel*

Culture vessel	Amount of DNA [µg]	Amount of siRNA [pmoles] 10 nM	Amount of siRNA [pmoles] 50 nM	Volume of jetPRIME® reagent [mL]	Volume of jetPRIME® buffer for complex formation [µL]	Volume of growth medium [mL]	Final volume in the well [mL]
24-well	0.5	5.5	27.5	1 – 1.5	50	0.55	0.55
12-well	1	11	55	2 – 3	100	1	1.1
6-well/35 mm	2	22	110	4 – 6	200	2	2.2
60 mm/flask 25 cm²	4	42	210	8 – 12	200	4	4.2
100 mm/flask 75 cm²	10	105	525	20 – 30	500	10	10.5

4 Transfection of CRISPR/Cas9

jetPRIME® is well-suited for genome editing using the CRISPR/Cas9 technology which can be achieved by transient transfection of different types of nucleic acids into mammalian cells:

- One single DNA plasmid containing sequences for both Cas9 nuclease and a non-coding guide RNA (gRNA),
- Multiple DNA plasmids (separate plasmids coding for Cas9, gRNA and potentially a sequence to be inserted in the genome)
- A mix of one or two DNA plasmids and a RNA molecule (the gRNA).

For co-transfection of multiple nucleic acids, the total DNA or nucleic acid amount per well (or plate) should not exceed the DNA amount indicated in Table 2. The optimal ratio of each nucleic acid in the transfection mix depends on the plasmid size, the constructs and the desired expression level for each nucleic acid. This ratio has to be optimized according to your application. Each plasmid or RNA molecule should represent at least 10% of the total nucleic acid amount per well/plate.

The following conditions are given per well of a 6-well plate. For other culture format, please refer to Table 2. For optimization, please refer to Table 3.

1. Dilute 2 µg total nucleic acid amount into 200 µL jetPRIME® buffer (supplied). Mix by vortexing.
2. Vortex jetPRIME® reagent for 5 seconds and spin down before use.
3. Add 4 µL jetPRIME®, vortex for 1 second, spin down briefly.
4. Incubate for 10 minutes at room temperature.
5. Add the transfection mix to the well dropwise onto the cells in serum containing medium and distribute evenly.
6. Gently rock the dish back and forth and from side to side, incubate the plate at 37 °C.
7. Eventually replace transfection medium after 4 hours by cell growth medium and return the plate to the incubator.

In case of enrichment for cells expressing CRISPR/Cas9 system based on an antibiotic selection, a selective amplification of targeted clones is performed by adding the antibiotic 24 to 48 hours post-transfection.

5 Stable DNA Transfection

jetPRIME® is suitable for stable DNA transfection.

Perform stable transfection in 6-well plates, 60 mm or 100 mm dishes.

1. If needed, linearize plasmid DNA construct encoding for antibiotic selection.
2. Perform transfection using the standard protocol described in Section 1.2.
3. Start antibiotic selection 24 to 48 hours after transfection.
4. Maintain antibiotic selection as long as required.
5. Check for integration of the plasmid DNA or stable expression of your protein of interest.

6 Troubleshooting

Observations	Actions
Low DNA transfection efficiency	• Optimize the volume of jetPRIME® reagent and the amount of plasmid DNA added per well. Increase the volume of jetPRIME® reagent first; if insufficient, increase the amount of DNA according to Table 3 • Ensure that transfection was performed in presence of serum since jetPRIME® gives higher transfection efficiency in presence of serum • Ensure the cells are NOT in Opti-MEM™ during transfection • Ensure that the nucleic acid is diluted in the provided jetPRIME® buffer • Use a plasmid containing a common reporter gene such as Luciferase or GFP as a positive control • Preferably use a DNA preparation at a concentration of 0.3 to 1 µg/µL • For cells known to be difficult to transfect, start by using 1.5x the amount of DNA suggested in Table 2. Then decrease the DNA amount if you observe toxicity • Use high-quality plasmid preparation, free of proteins and RNA ($OD_{260/280}$ >1.8) • Homogenize the transfection mix by pipetting up and down slowly instead of vortexing
Low siRNA mediated silencing efficiency	• Ensure that all reagents are RNAse free • Ensure that the quality of your siRNA is optimal (concentration, annealing and design) • Optimize the amount of siRNA and DNA used if performing co-transfection
Cellular toxicity	• Analyze transfection at an earlier time point (e.g. at 24 hours instead of 48 hours) • Wash cells 4 hours after transfection • Decrease the amount of plasmid DNA added per well • Ensure that the nucleic acid is diluted in the provided jetPRIME® buffer • Decrease the volume of jetPRIME® reagent • Ensure that the plasmid preparation is endotoxin-free • Verify the toxicity of the expressed protein. If the expressed protein is toxic for the cells, reduce the amount of plasmid DNA

7 Product Information

7.1 Ordering Information

Part number	jetPRIME® reagent [mL]	Buffer [mL]
101000027	0.1	5
101000015	0.75	60
101000046	1.5	2x60
101000001	5x1.5	10x60

7.2 Additional Buffer

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

7.3 Content

1.5 mL of jetPRIME® transfection reagent is sufficient to perform up to 1,500 transfections in 24-well plates or 375 transfections in 6-well plates.

7.4 Reagent Use and Limitations

For research use only. Not for use in humans.

7.5 Quality Control

Every batch of jetPRIME® reagent is tested by DNA transfection of HeLa cells with a GFP-expressing plasmid. Certificates of Analysis are available online in the MySartorius portal on www.sartorius.com.

7.6 Formulation and Storage

jetPRIME® and its buffer should be stored at 4°C upon arrival to ensure long term stability. jetPRIME®, as guaranteed and indicated on the Certificate of Analysis, is stable at least for 6 months (101000027) to at least one year (other packaging sizes) when stored appropriately.

7.7 Trademarks

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