

jetPRIME®

in vitro DNA & siRNA transfection reagent

PROTOCOL

DESCRIPTION

jetPRIME® is a novel powerful transfection reagent based on a polymer formulation manufactured at Polyplus-transfection®. jetPRIME® ensures 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**.

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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 h prior to transfection. For other culture formats, refer to Table 1.

jetPRIME® is stable in presence of serum and antibiotics therefore you may use serum and antibiotic containing medium during the entire experiment.

Table 1. 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)
96-well	7 500 - 10 000	0.3	0.1
24-well	50 000 - 80 000	1.9	0.5
12-well	80 000 - 150 000	3.8	1
6-well / 35 mm	150 000 - 250 000	9.4	2
60 mm / flask 25 cm ²	250 000 - 800 000	25 - 28	5
100 mm / flask 75 cm ²	1 x 10 ⁶ - 2 x 10 ⁶	75 - 78.5	10
150 mm / flask 175 cm ²	2 x 10 ⁶ - 5 x 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. Add 4 µl jetPRIME®, vortex for 10 s, spin down briefly.
3. Incubate for 10 min at RT.
4. Add 200 µl of transfection mix per well drop wise onto the cells in serum containing medium, and distribute evenly.
5. Gently rock the plates back and forth and from side to side.
6. If needed, replace transfection medium after 4 h by cell growth medium and return the plates to the incubator.
7. Analyze after 24 h or later.

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

Culture Vessel	Volume of jetPRIME® Buffer (µl)	Amount of DNA (µg)	Volume of jetPRIME® reagent (µl)	Volume of growth medium (ml)
96-well*	5	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 cm ²	200	4	8 - 12	5
100 mm / flask 75 cm ²	500	10	20 - 30	10
150 mm / flask 175 cm ²	1000	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.

Browse our cell transfection database to find the optimized conditions according to your cell line.

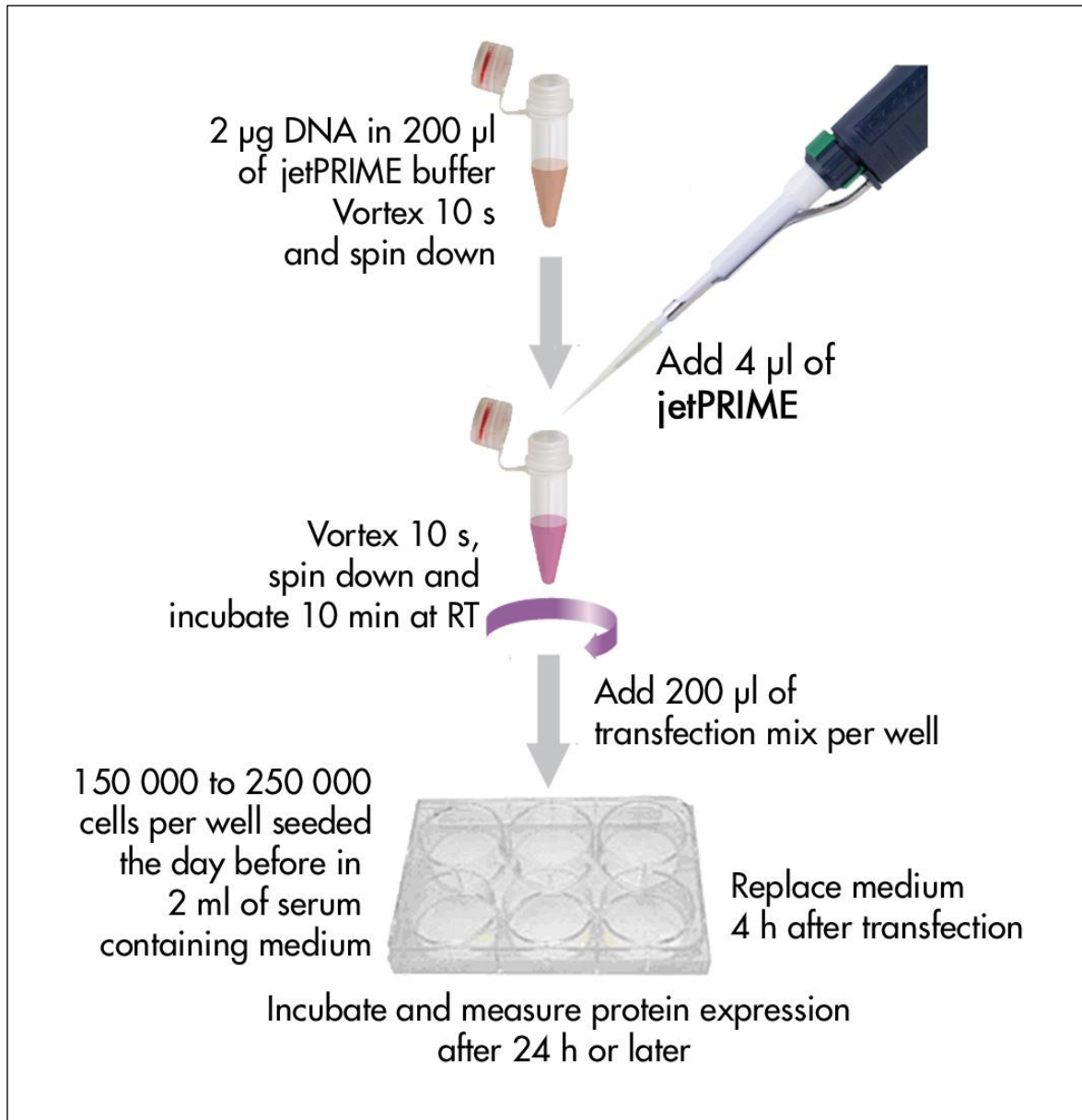
<http://www.polyplus-transfection.com/resources/cell-transfection-database/>



Watch the video "DNA Transfection using jetPRIME®" on YouTube!

<http://www.youtube.com/watch?v=G39wNX>

DNA Transfection in 6-well plates



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 cotransfection 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. Add 20 µl jetPRIME®, vortex for 10 s, spin down briefly.
3. Incubate for 10 min at RT.
4. Add 500 µl of transfection mix per dish drop wise onto the cells in serum containing medium, and distribute evenly.
5. Gently rock the dish back and forth and from side to side.
6. Eventually replace transfection medium after 4 h by cell growth medium and return the dish to the incubator.
7. Incubate 24 to 72 h and proceed to virus purification and titration.

1.4 OPTIMIZATION GUIDELINES

Transfection conditions should be optimized for each tested cell line according to the conditions detailed below (Table 3). Specific conditions for many cell lines can be found in our cell transfection database, following this link: <http://www.polyplus-transfection.com/resources/cell-transfection-database/>

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 0.5 X and 1.5 X, X being the amount 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 0.5 X (Table 3).

Table 3: Transfection optimization guidelines

Culture Vessel	Volume of jetPRIME® Buffer (µl)	Amount of DNA (µg)	Volume of jetPRIME® reagent (µl)
96-well*	5	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 / 3.5 cm	200	1 - 3	2 - 9
60 mm / flask 25 cm ²	200	2 - 6	4 - 18
100 mm / flask 75 cm ²	500	5 - 15	10 - 45
150 mm / flask 175 cm ²	1000	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 h prior to transfection. For other culture formats, refer to Table 4.

jetPRIME® is stable in the presence of serum and antibiotics therefore you may use serum and antibiotic containing medium during the entire experiment.

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	25 000 - 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	5
100 mm / flask 75 cm ²	0.5 x 10 ⁶ - 1 x 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 pipeting up and down.
2. Add 4 µl jetPRIME® reagent, vortex for 10 s, spin down briefly.
3. Incubate for 10 to 15 min at RT.
4. Add the transfection mix to the cells in serum containing medium drop wise.
5. Gently rock the plate back and forth and return the plate to the incubator.
6. If needed, replace transfection medium by cell growth medium 24 h 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 & siRNA COTRANSFECTION PROTOCOL

3.1 CELL SEEDING

For optimal cotransfection 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 h prior to transfection. For other culture formats, refer to Table 1.

jetPRIME® is stable in the presence of serum and antibiotics therefore you may use serum and antibiotic containing medium during the entire experiment.

3.2 DNA & siRNA COTRANSFECTION PROTOCOL

For DNA/siRNA cotransfection 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 pipeting up and down.
2. Add 4 µl jetPRIME® reagent, vortex for 10 s, spin down briefly.
3. Incubate for 10 to 15 min at RT.
4. Add the transfection mix to the cells still in serum containing medium drop wise.
5. Gently rock the plate back and forth and return the plate to the incubator.
6. If needed, replace transfection medium by cell growth medium 24 h 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 & siRNA cotransfection 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 (µl)	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 cm ²	4	42	210	8 - 12	200	4	4.2
100 mm / flask 75 cm ²	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 cotransfection 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. Add 4 µl jetPRIME®, vortex for 10 s, spin down briefly.
3. Incubate for 10 min at RT.
4. Add the transfection mix to the well drop wise onto the cells in serum containing medium, and distribute evenly.
5. Gently rock the dish back and forth and from side to side.
6. Eventually replace transfection medium after 4 h 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 h to 48 h 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 h 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 OptiMEM® 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 1.5 x 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).
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 cotransfection.
Cellular toxicity	Analyze transfection at an earlier time point (e.g. at 24 h instead of 48 h).
	Wash cells 4 h 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

Cat. N°	jetPRIME® Reagent	jetPRIME® Buffer
114-01	0.1 ml	5 ml
114-07	0.75 ml	60 ml
114-15	1.5 ml	2 x 60 ml
114-75	5 x 1.5 ml	10 x 60 ml
114-75C	5 x 1.5 ml	120 ml 5X CONC.

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. The 5X concentrated buffer provided with catalogue number 114-75C needs to be diluted 1:5 in sterile ddH₂O before use.

7.3 CONTENT

1.5 ml of jetPRIME® transfection reagent is sufficient to perform up to 1500 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.

7.6 FORMULATION AND STORAGE

jetPRIME® and its buffer are shipped at room temperature but 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 for 6 months (114-01) to at least one year (other packaging sizes) when stored appropriately.

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.

7.7 TRADEMARKS

Polyplus-transfection and jetPRIME are registered trademarks of Polyplus-transfection.

7.8 TECHNICAL ASSISTANCE AND SCIENTIFIC ADVICE

Contact the friendly Polyplus technical support *via*:

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