

***In vitro* DNA & siRNA Transfection Protocol**

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Company Information

Technical Assistance and Scientific Advice

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Product Information

jetPRIME™ is a novel powerful molecule based on a non-liposomal formulation that ensures effective and reproducible DNA and siRNA transfection into mammalian cells. jetPRIME™ is **extremely efficient** on a variety of cell lines. It is very powerful and only requires low amounts of nucleic acid per transfection; hence resulting in **very low cytotoxicity**. jetPRIME™ is manufactured at Polyplus-transfection.

Ordering information

Cat #	jetPRIME™ Reagent	jetPRIME™ Buffer
114-01	0.1 ml	5 ml
114-07	0.75 ml	40 ml
114-15	1.5 ml	2 x 40 ml

Additional Buffer

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

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.

Reagent use and Limitations

For research use only. Not for use in humans.

Quality control

Every batch of jetPRIME™ reagent is tested by DNA transfection of HeLa cells with a GFP expressing plasmid. The transfection efficiency value for each batch is indicated on the Certificate of Analysis.

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 by the Certificate of Analysis, will be stable for at least one year when stored appropriately.

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 for the recommended number of cells to seed the day before transfection and the recommended volume to seed the cells in.

jetPRIME™ is stable in the 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/3.5 cm	150 000 – 250 000	9.4	2
6 cm/flask 25 cm ²	250 000 – 800 000	25 - 28	5
10 cm/flask 75 cm ²	1 x 10 ⁶ – 2 x 10 ⁶	75 - 78.5	10
14 cm/flask 175 cm ²	2 x 10 ⁶ – 5 x 10 ⁶	153 - 175	20

1.2 DNA Transfection Protocol

a. Overview and guidelines for any cell culture vessel format

1. Dilute DNA in jetPRIME™ buffer*, vortex, spin down.
2. Add jetPRIME™ reagent, vortex, spin down.
3. Incubate for 10 min at RT.
4. Add a fraction or the entire transfection mix to the cells according to Table 2.
5. Change medium after 4h if needed and analyze after 24h.

*jetPRIME™ buffer must be used for successful transfection.

Table 2. DNA Transfection guidelines according to the cell culture vessel

Culture vessel	Transfection Mix			Per Well		
	Volume of jetPRIME™ Buffer (µl)	Amount of DNA (µg)	Volume of jetPRIME™ reagent (µl)	Volume of growth medium (ml)	Volume of transfection mix (µl)	Final maximal DNA amount (µg)
96-well	100	1	2	0.1	5	0.05
24-well	100	1	2	0.5	50	0.5
12-well	150	1.5	3	1	75	0.75
6-well / 3.5 cm	200	2	4	2	200	2
6 cm / flask 25 cm ²	200	4	8	5	200	4
10 cm/ flask 75 cm ²	500	10	20	10	500	10
14 cm/ flask 175 cm ²	1000	20	40	20	1000	20

A minimal volume of 100 µl transfection mix should be prepared to ensure homogenous preparation of the complexes.

Standard conditions: **1:2 DNA to jetPRIME™ ratio (w/v)**

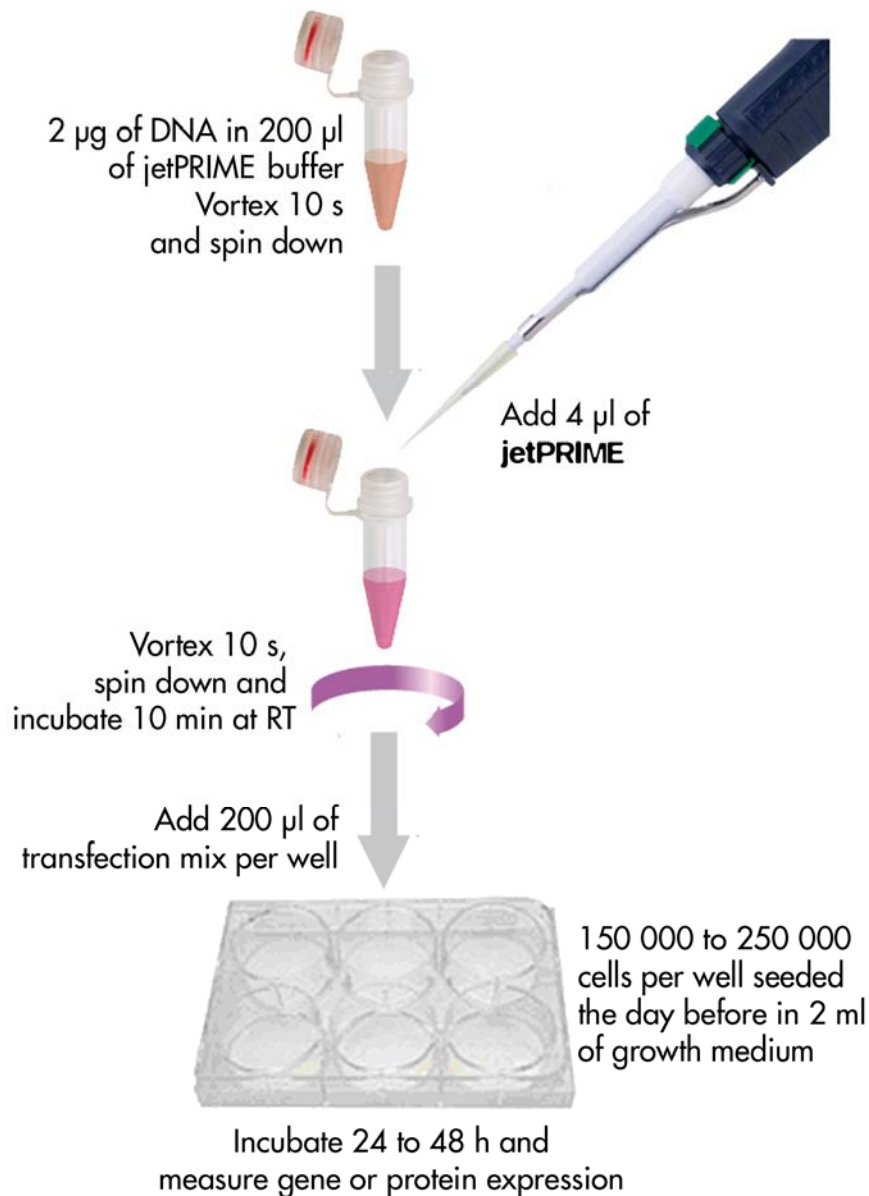
i.e. for 1 µg of DNA use 2 µl of jetPRIME™.

Due to the high performance of jetPRIME™ reagent, the amount of plasmid DNA may be decreased, as indicated in Table 3; the less DNA, the lower the toxicity.

b. Detailed protocol in 6-well plates/3.5 cm dishes

1. Dilute 2 µg DNA into 200 µl jetPRIME™ buffer (supplied). Mix by vortexing.
2. Add 4 to 6 µl jetPRIME™, vortex for 10 s.
3. Incubate for 10 min at RT.
4. Add 200 µl transfection mix per well drop wise onto the cells to distribute evenly.
5. Gently rock the plates back and forth and from side to side.
6. Replace transfection medium after 4 h by 4 ml of growth medium and return the plates to the incubator.
7. Analyze after 24 h or later

DNA Transfection in 6-well plates



c. Optimization guidelines

Transfection conditions should be optimized for each tested cell line according to the conditions detailed below (Table 3). You may adjust the volume of reagent and/or the amount of DNA added per well.

The amount of jetPRIME™ may range between 2 to 4 µl per µg of DNA depending on the transfected cell line.

Due to the high performance of jetPRIME™ reagent, you may decrease the amount of plasmid DNA by adjusting the volume of transfection mix added per well.

Table 3. Transfection optimization guidelines.

Culture vessel	Transfection Mix			Added per Well	
	Volume of jetPRIME™ Buffer (µl)	Amount of DNA (µg)	Volume of jetPRIME™ reagent (µl)	Volume of transfection mix (µl)	Final DNA amount (µg)
96-well	100	1	2 – 4	2.5 – 10	0.025 – 0.1
24-well	100	1	2 – 4	25 – 50	0.25 – 0.5
12-well	150	1.5	3 – 6	50 – 75	0.5 – 0.75
6-well / 3.5 cm	200	2	4 – 8	100 – 200	1 – 2
6 cm / flask 25 cm ²	200	2 – 5	4 – 20	100 – 200	2 – 5
10 cm / flask 75 cm ²	500	5 – 15	10 – 60	500	5 – 15
14 cm / flask 175 cm ²	1000	10 – 30	20 – 120	1000	10 – 30

2. siRNA transfection

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 for the recommended number of cells to seed the day before transfection and the recommended volume to seed the cells in.

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 / 3.5 cm	100 000 – 150 000	9.4	2
6 cm / flask 25 cm ²	200 000 – 500 000	25 - 28	5
10 cm / flask 75 cm ²	1 x 10 ⁶ – 2 x 10 ⁶	75 - 78.5	10
14 cm / flask 175 cm ²	2 x 10 ⁶ – 5 x 10 ⁶	153 - 175	20

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 μ l of jetPRIME™ buffer. Mix by pipeting up and down.
2. Add 4 μ l jetPRIME™ reagent, vortex for 10 sec, spin down briefly.
3. Incubate for 10 to 15 min at RT.
4. Add the transfection mix to the cells drop wise.
5. Gently rock the plate back and forth and return the plate to the incubator.
6. Replace transfection medium by cell growth medium 24 hours after transfection and analyse 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 (μ l)	Volume of jetPRIME™ Buffer for complex formation (μ l)	Volume of growth medium per well	Final volume in the well
24-well	5.5	27.5	2	50*	500 μ l	550 μ l
12-well	11	55	3	100	1 ml	1.1 ml
6-well / 3.5 cm	22	110	4	200	2 ml	2.2 ml
6 cm / flask 25 cm ²	42	210	8	200	4 ml	4.2 ml
10 cm / flask 75 cm ²	105	525	20	500	10 ml	10.5 ml

* A minimal volume of 100 μ l transfection mix should to be prepared to ensure homogenous preparation of the complexes.

3. DNA & siRNA co-transfection

3.1 Cell seeding

For optimal 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 for the recommended number of cells to seed the day before transfection and the recommended volume to seed the cells in.

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 co-transfection protocol

For DNA/siRNA co-transfection experiment, we recommend using 400 ng 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 format, please refer to Table 6.

1. Dilute 400 ng 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 sec.
3. Incubate for 10 to 15 min at RT.
4. Add the transfection mix to the cells drop wise.
5. Gently rock the plate back and forth and return the plate to the incubator.
6. Replace transfection medium by cell growth medium 24 hours after transfection and analyze as required.

Table 6. DNA & siRNA co-transfection guidelines according to the cell culture vessel.

Culture vessel	Amount of DNA (ng)	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 on the cells	Final volume in the well
24-well	100 -200 ng	5.5	27.5	2	50*	500 µl	550 µl
12-well	150-250 ng	11	55	3	100	1 ml	1.1 ml
6-well / 3.5 cm	400 ng	22	110	4	200	2 ml	2.2 ml
6 cm/ flask 25 cm ²	800 ng	42	210	8	200	4 ml	4.2 ml
10 cm/ flask 75 cm ²	2 µg	105	525	20	500	10 ml	10.5 ml

* A minimal volume of 100 µl should to be prepared to ensure homogenous preparation of the complexes.

4. Stable DNA transfection

jetPRIME™ is suitable for stable DNA transfection.

Perform stable transfection in 6-well plates, 60 mm or 10 cm dishes.

1. If needed, linearize plasmid DNA construct encoding for antibiotic selection.
2. Perform transfection as described in the standard protocol in Section 1.2.
3. Start antibiotic selection 24 – 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 expression.

Contact our Technical Assistance and Scientific Advice Service

Contact the friendly Polyplus technical support *via*:

The Polyplus website: www.polyplus-transfection.com

Email: support@polyplus-transfection.com

Phone: + 33 (0) 3 90 40 61 87

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™ reagents first; if insufficient, increase the amount of DNA.
	Use high-quality plasmid preparation, free of proteins and RNA ($OD_{260/280} > 1.8$).
	Use a plasmid containing a common reporter gene such as Luciferase or GFP as 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 double the amount of DNA than suggested in Table 3. Then decrease DNA amount.
	Ensure that jetPRIME™ reagent was diluted in jetPRIME™ buffer included and not in any other buffer.
Low siRNA mediated silencing efficiency	Ensure that all reagents are RNase free.
	Ensure of the quality of your siRNA (desalted, concentration, annealing, design).
	Optimize the amount of siRNA used and of DNA if performing co-transfection.
Cellular toxicity	Analyze transfection at an earlier time point e.g. at 24 h instead of 48 h.
	Ensure that jetPRIME™ reagent was diluted in jetPRIME™ buffer included.
	Decrease the amount of plasmid DNA by decreasing the volume of transfection mix added to the cells.
	Verify the toxicity of the expressed protein. If the expressed protein is toxic for the cells, reduce the amount of plasmid DNA.
	Decrease the amount of jetPRIME™ reagent.
	Ensure that the plasmid preparation is endotoxin-free.

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