Cat # SL100688 Store at 4 °C

PolyJet™ In Vitro DNA Transfection Reagent

----- A General Protocol for Transfecting Mammalian Cell

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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This product is for laboratory research ONLY and not for diagnostic use

Introduction:

Based on our innovative polymer synthesis technology, PolyJet™ DNA In Vitro Tranfection Reagent is formulated to be a powerful transfection Reagent that ensures effective and reproducible transfection with less cytotoxicity. PolyJet™ was shown to deliver genes to various established cell lines as well as primary cells.

Important Guidelines for Transfection:

- PolyJet™ reagent was formulated for DNA transfection ONLY! The following standard protocol is for transfecting mammalian cells. To request protocol for lentivirus, rAAV or adenovirus production, please email us at info@signagen.com
- For better efficiency, choosing a correct protocol is essential. We strongly encourage to use "General Protocol" first. If the "General Protocol" fails to give satisfactory result (e.g., less than 10%), try the "Advanced Protocol" in the back page
- For high efficiency and lower toxicity, transfect cells at high density. 70~80% confluency is highly recommended
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics

Part I. A General Procedures for Transfecting Adherent Cells

Step I. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 70~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. We recommend using complete serum/antibiotics-containing medium as a starting point. For maximal efficiency and lower cytotoxicity, perform transfection on cells with high density. We recommend transfecting on cells with ~80% confluency.

Step II. Preparation of PolyJet™-DNA Complex and Transfection Procedures:

For different cell types, the optimal ratio of PolyJet™ (µL):DNA (µg) is around 3:1. We recommend the PolyJet™ (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of PolyJet™/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and PolyJet™ Reagent.

The following protocol is given for transfection in 24-well plates, refer to **Table 1** for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines, as well as a general starting point for optimization are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each well, dilute 0.5 µg of DNA into 25 µl of serum-free DMEM with High Glucose. Gently pipette up and down or vortex briefly to mix.
- For each well, dilute 1.5 µl of PolyJet™ reagent into 25 µl of serum-free DMEM with High Glucose. Gently pipette up and down 3~4 times to mix.

  Note: Never use Opti-MEM to dilute PolyJet™ reagent and DNA, it contains serum and will disrupt transfection complex.
- Add the diluted PolyJet™ reagent **immediately** to the diluted DNA solution all at once. (**Important: do not mix the solutions in the reverse order !**)
- Immediately pipette up and down 3~4 times or vortex briefly to mix.
- Incubate for 10~15 minutes at room temperature to allow PolyJet™/DNA complexes to form.

  Note: Never keep the PolyJet™/DNA complex longer than 20 minutes.
- Add the 50 µl PolyJet™/ DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Remove PolyJet™/DNA complex-containing medium and replace with fresh complete serum/antibiotics containing medium 12~18 hours post transfection. **For sensitive cells, to lower cytotoxicity, remove PolyJet™/DNA complex and replace with complete medium 5 hours after transfection.**
- Check transfection efficiency 24 to 48 hours post transfection.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	PolyJet™ Reagent (µL)
48 well plate	0.3	0.25	2 x 0.015	0.75
12 well plate	0.75	0.75	2 x 0.038	2.25
6-well plate	1.0	1	2 x 0.05	3.0
35 mm dish	1.0	1	2 x 0.05	3.0
60 mm dish	2.8	2.5	2 x 0.10	7.5
10 cm dish	5.0	5	2 x 0.25	15
T75 flask	8.0	9 - 18	2 x 0.40	27 - 54
250 ml flask	18	25 - 50	2 x 0.8	75 - 150

Storage: PolyJet™ Reagent is stable for up to 12 months at +4 °C after receipt

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PolyJet™ In Vitro DNA Transfection Reagent

----- An Advanced Protocol for Transfecting Hard-to-Transfect Cells

☐ 100 µl
☐ 500 µl
☐ 1000 µl

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Part II. Advanced Protocol for Transfecting Hard-To-Transfect Mammalian Cells

Important: The advanced protocol for hard-to-transfect cells is provided only if general protocol gives less than 10% efficiency. **For some primary cells which cannot be trypsinized (like primary neurons), go directly to Step II, skip trypsinization and incubate freshly prepared primary cell pellet with transfection complex.**

Step I. Culturing of Cells Before Transfection:

Cells should be plated at least 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluency at the day of transfection.

Table 2. A Guideline for Optimal Cell Number Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm^2)	Optimal Cell Number
T75 Flask	75	9.6×10^6
100 mm Dish	58	7.3×10^6
60 mm Dish	21	2.7×10^6
35 mm Dish	9.6	1.0×10^6
6-well Plate	9.6	1.0×10^6
12-well Plate	3.5	0.44×10^6
24-well Plate	1.9	0.24×10^6
48-well Plate	1.0	0.11×10^6
96-well Plate	0.3	0.31×10^5

Table 3. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Complex Volume (ml)	Plasmid DNA (µg)	PolyJet™ Reagent (µL)
96-well	0.02	0.2	0.8
48-well	0.04	0.5	2
24-well	0.1	1	4
12-well	0.12	1.2	4.8
6-well	0.2	2	8
35 mm dish	0.2	2	8
60 mm dish	0.5	5	20
10 cm dish	1.0	8	32
T75 flask	1.5	36	144
250 ml flask	2.5	100	400

Step II. Preparation of Cells in Suspension:

The following protocol is given for transfecting hard-to-transfect cells in 6-well plates, refer to Table 2 for optimal cell number per well per Culture vessels' surface area. The optimal transfection conditions are given in the standard protocol described below.

- Detach the cells with trypsin/EDTA and stop the trypsinization with complete culture medium.

  Note: Cells that are difficult to detach may be placed at 37 °C for 5-15 min to facilitate detachment
- Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
- Centrifuge the required ~1.0×10^6 cells per well for 6-well plate at 150xg at room temperature for 10 min.
- Use fine tip pipette to remove supernatant **completely** so that no residual medium covers the cell pellet.

Step III. Preparation and application of Transfection Complex:

For most of mammalianells, the optimal ratio of PolyJet™ (µL):DNA (µg) is 4:1. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and PolyJet™ Reagent.

The following protocol is given for transfection in 6-well plates, refer to **Table 3** for transfection in other culture formats.

- For each well of 6-well plate, dilute 2 µg of DNA into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube.
- For each well of 6-well plate, dilute 8 µl of PolyJet™ reagent into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the diluted PolyJet™ Reagent immediately to the diluted DNA solution all at once. **(Important: do not mix the solutions in the reverse order !)**
- Immediately pipette up and down 3~4 times or vortex briefly to mix followed by incubation for ~15 minutes at room temperature to allow PolyJet™/DNA transfection complexes to form.

  Note: Never keep the transfection complexes longer than 20 minutes
- **Gently** resuspend the cell pellet prepared from **Step II** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 minutes.
- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate. Incubate at 37 °C with 5% CO_2.
- Remove transfection complex containing medium **gently** and refill with complete culture medium 8~12 hours after plating.
- Check transfection efficiency 24 to 48 hours post transfection.