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 production and insect cells transfection, 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. Vortex gently and spin down briefly to bring drops to the bottom of the tube.
- For each well, dilute 1.5 µl of PolyJet™ reagent into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to the bottom of the tube.

  Note: Never use Opti-MEM to dilute PolyJet™ reagent and DNA, it 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 ~15 minutes at room temperature to allow PolyJet™/DNA complexes to form.

  Note: Never keep the PolyJet™/DNA complex longer than 30 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	3 - 4	2 x 0.25	9 - 12
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