

# 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 Transfection 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](mailto: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

