Cat # SL100688 Store at 4 °C

PolyJet™ In Vitro DNA Transfection Reagent

----- A Protocol for Generation of rAAV from 293T 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™ reagent was shown to deliver DNA to various established cell lines as well as primary cells. PolyJet™ reagent was shown to generate rAAV with extremely high titers from 293T cells.

Important Transfection Guidelines:

- For high titer of rAAV, 293T cell must be healthy. Please grow the 293T cell per supplier's instruction.
- For high efficiency, transfect cells at high density. ~80% confluency is highly recommended.
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.
- Use serum-free DMEM with High Glucose to dilute PolyJet™ reagent and DNA. The diluent must be serum-free.

Procedures for Transfecting 293T Cells:

Cell Seeding (see Table 1):

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~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. For some specific 293 cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.

Table 1. A Guideline for Seeding 293T Cells Prior to Transfection in Different Culture Formats.

Culture Dishes	Surface Area (cm2)	Number of Cells to Seed
T75 Flask	75	3.0 – 6.0 x 10^6
100 mm Dish	58	2.2 – 4.4 x 10^6
60 mm Dish	21	0.9 – 1.8 x 10^6
35 mm Dish	9.6	3.5 – 7.0 x 10^5
6-well Plate	9.6	4.0 – 8.0 x 10^5
12-well Plate	3.5	1.5 – 3.0 x 10^5
24-well Plate	1.9	0.8 – 1.6 x 10^5
48-well Plate	1.0	4.0 – 8.0 x 10^4

Preparation of PolyJet™ -DNA Complex and Transfection Procedures

The following protocol is given for transfection in 150 mm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

- Cell confluency should be ~80 % at the day of transfection
- For each 15 cm dish, add 18 mL of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each dish, dilute 5 µg of rAAV cis plsamid, 5 µg capsid DNA and 8 µg helper DNA (total 18 µg DNA) in 1000 µL serum-free DMEM with high glucose. Vortex gently to mix.
- Add 54 µL of PolyJet™ reagent into the above viral plasmid-containing serum-free DMEM with high glucose. Vortex gently to mix.

  Note: Never use Opti-MEM to dilute DNA and PolyJet™ reagent because it will disrupt transfection complex.
- Incubate the transfection mix for 10 min at room temperature to allow DNA-PolyJet™ transfection complex to form.

  Note: Never keep the DNA/PolyJet™ complex longer than 20 min
- Add PolyJet™/DNA complex drop-wise onto the medium in each dish and homogenize the mixture by gently swirling the plate.
- Remove DNA/PolyJet™ complex-containing medium and replace with fresh complete serum/antibiotics containing medium 24 hours post transfection.
- Harvest the AAV 72 hours post transfection.

Storage: PolyJet™ DNA In Vitro Transfection Reagent is stable for up to 24 months at +4 °C. This item shipped at ambient temperature