Cat # SL100688 Store at 4 °C

# PolyJet™ DNA In Vitro Transfection Reagent (Ver. II)

## -----A Protocol for Transfecting 293 and CHO Cells in Suspension

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
9061 Medical Center Drive
A/R Building, Suite 341
Rockville MD 20850
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

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

**Introduction:**
Based on our proprietary polymer synthesis technology, PolyJet™ DNA In Vitro Transfection Reagent is formulated as a biodegradable polymer based DNA transfection reagent that ensures effective and reproducible transfection on HEK293, COS-7, NIH-3T3, HeLa, CHO and a broad ranges of hard-to-transfect mammalian cells. A remarkable feature of the reagent is the rapid and complete degradation of polymer after transfection complex endocytosis, leading to much less cytotoxicity.

**Important Guidelines for Transfection:**
- PolyJet™ reagent was formulated for DNA transfection ONLY! The following standard protocol is for transfecting suspension 293 or CHO cells.
- To request protocol for lentivirus, rAAV an adenovirus production, please email us at info@signagen.com or visit our website at www.signagen.com

**Advantages:**
Using PolyJet™ Reagent provides the following advantages:
- PolyJet™ Reagent demonstrates high transfection efficiency in both suspension and adherent 293 cells.
- Add PolyJet™ Reagent/DNA complexes directly to cells in standard culture medium and no medium change is required.

**Important Guidelines for Transfection:**
- For optimal transfection efficiency, dilute PolyJet™ Reagent and plasmid in serum-free DMEM prior to the formation of transfection complex.
- Make sure your plasmid DNA is in high quality and clean and sterile without contamination of phenol and salt.

**Recommended Conditions for Transfection:**
To transfect suspension 293 and CHO cells in their standard cuture medium, use the following optimized transfection conditions. To perform transfection experiments in a larger volume, simply scale up the volume of reagents accordingly.
- Final transfection volume: 32 mL.
- Number of cells to transfect: 3E+7 cells at final cell density of 1E+6 cells/mL cultured in standard culture medium. Make sure that the cells are healthy and greater than 90% viable before transfection.
- Amount of plasmid DNA: ~25 µg.
- Amount of PolyJet™ Reagent: ~60 µL. Lock the ratio of PolyJet™ Reagent/DNA at 2.4:1.

**Procedures for Transfecting Suspension 293 or CHO Cells:**
Follow the procedure below to transfect suspension 293 or CHO cells in a 30 ml volume. If you wish to transfect the suspension cells in a larger volume, scale up the transfection condistions in proportion to the culture volume.
- The day before transfection, determine the numbers of the cells and grow suspension 293 or CHO cells so that at the day of transfection (roughly 24 hours after) the cell density reaches 3E+7 cells in total 30 mL standard culture medium.
- At the day of transfection, count cell viability and adjust cell density at 1.0E+6 per mL in total 30 mL (total 3E+7 cells) standard culture medium. Place the shaker flask containing cells in a 37°C incubator on an orbital shaker.

  **Important:** For best results, make sure to have a single-cell suspension. It may be necessary to vortex the cells vigorously for 10-30 seconds to break down cell clumps. The viability of cells must be >90%.
- For each transfection, prepare lipid-DNA complexes as follows:
  1). Dilute 25 µg of plasmid DNA in serum free DMEM to a total volume of 1 mL. Vortex to mix.
  2). Dilute 60 µL of PolyJet™ Reagent in serum free DMEM to a total volume of 1 mL. Vortex to mix.

  **Note:** Never use Opti-MEM to diliute plasmid and PolyJet™ Reagent because trace of serum from Opti-MEM may interfere formation of lipid-DNA complex.
  3). Add diluted PolyJet reagent to the diluted DNA right away at all once to obtain total volume of 2 mL transfection mix. Vortex to mix.
  4). Incubate for 10 minutes at room temperature to allow the formation of DNA-PolyJet™ complexes.

  **Important:** Never leave the DNA-PolyJet complex longer than 20 minutes at RT before addition to suspension 293 or CHO cells.
- Add the 2 mL of DNA-PolyJet™ complex to each shaker flask containing 30-mL suspension 293 or CHO cells.
- Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO2 in air on an orbital shaker rotating at 125 rpm.
- Harvest cells or media (if recombinant protein is secreted) at around 48 hours post-transfection and assay for recombinant protein expression.

**Storage:** PolyJet™ DNA In Vitro Transfection Reagent (Ver. II) is stable for up to 12 months at 4 °C. This item shipped at ambient temperature