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PeneFect™ Transfection Reagent

Cat. #: M0001 Size: 1 ml/5 ml

Protocol for Transfecting 293 and CHO Cells in Suspension

Introduction:

Based on our proprietary polymer synthesis technology, PeneFect™ Plus In Vitro DNA 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 remar-kable feature of the reagent is the rapid and complete degradation of polymer after transfection complex endocytosis, leading to much less cytotoxic-ity.

Important Guidelines for Transfection:

- For optimal transfection efficiency, dilute PeneFect™ 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.

- PeneFect™ 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 produc-tion, please email us at support@lifesct.com or visit our website at www.lifesct.com.

Key Features:

- PeneFect™ Reagent demonstrates high transfection efficiency in both suspension and adherent 293 cells.

- Add PeneFect™ Reagent/DNA complexes directly to cells in standard culture medium and no medium change is required.

Recommended Conditions for Transfection:

To transfect suspension 293 and CHO cells in their standard cuture medium, use the following optimized transfection condi-tions. 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 PeneFect™ Reagent: ~60 µl. Lock the ratio of PeneFect™ 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. 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 PeneFect™ Reagent in serum free DMEM to a total volume of 1 ml. Vortex to mix.

Note: Never use Opti-MEM to diliute plasmid and PeneFect™ Reagent because trace of serum from Opti-MEM may interfere formation of lipid-DNA complex.

3) Add diluted PeneFect™ 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/PeneFect™ 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/PeneFect™ complex to each shaker flask containing 30ml suspension 293 or CHO cells.

- Incubate the cells in a 37°C incubator with a humidified atmo-sphere 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: PeneFect™ Transfection Reagent is stable for up to 12 months at 4°C.

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