General Considerations for Transfection

Protocol

This protocol is for the General Considerations for Transfection

I. DNA Quality Requirements.
DNA quality is critical for successful transfection. Endotoxin-contaminated DNA may result in inefficient transfection and cause unacceptably high cellular toxicity. For DNA an A260/A280 ratio of 1.8, or greater is recommended.

II. Cell Density.
The recommended confluency for adherent cells on the day of transfection is 50-70% and 70-90% for TurboFect™ reagents. Suspension cells should be plated at an optimal density ensuring their logarithmic growth at the time of transfection.

III. Incubation Time.
Transient transgene expression takes place within 2-72 hours after DNA transfection. The optimal time depends on the cell type, promoter strength and expression product, and has to be determined experimentally. The recommended incubation time of cells with TurboFect™/protein complexes is 2 hours.

IV. Choice of Promoter.
High transfection efficiency depends both on the transgene promoter and on the cell line used. The cytomegalovirus (CMV) promoter is commonly used for high gene expression in a variety of cell lines. Other promoters, such as those from simian virus (SV40) and from Rous sarcoma virus (RSV) can also be used.

V. Transfection Reagent/Biomolecule Ratio.
The amount of transfection reagent used in transfection depends on the amount of DNA, siRNA or protein and cells to be transfected. The ratios presented in the protocols are starting ratios and can be further optimized for the best results.

VI. Transfection in the Presence of Serum.
Nucleic acid transfection efficiency using transfection reagents is consistently high in the presence of serum. The presence of serum may reduce protein transfection efficiency by up to 50%. Therefore, protein transfection in serum-free medium is recommended for best results.

VII. Centrifugation.
Gentle centrifugation of tissue culture plates for 5 minutes at 280 x g after addition of the polyplexes can improve transfection efficiency.

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