INTRODUCTION

NeoFectin® Transfection Reagent is a non-Liposomal, chemically-defined, high-performance, broad spectrum transfection reagent that provides highly efficient transfection of DNA/RNA into mammalian cells, including hard-to-transfect cell types, such as primary cells and stem cells. It is serum compatible, low in toxicity, and easy to use.

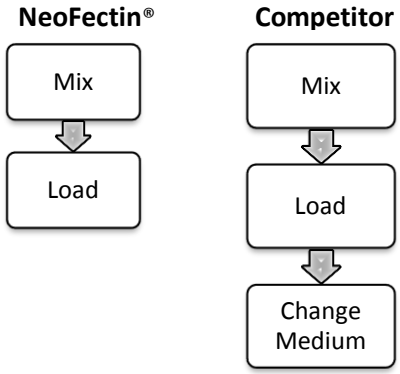
Catalog No.	Quantity
Trial sample	100 µl
NF00102	0.2 ml
NF00105	0.5 ml
NF00110	1.0 ml

Materials required, but not supplied
Mammalian cells
DNA or RNA
Cell culture medium
Sterile tubes
Micropipets
Cell culture vessels
Optional: Selection antibiotic

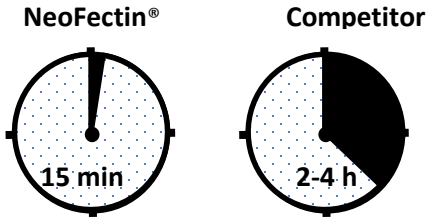
Storage: 4 degrees

FEATURES

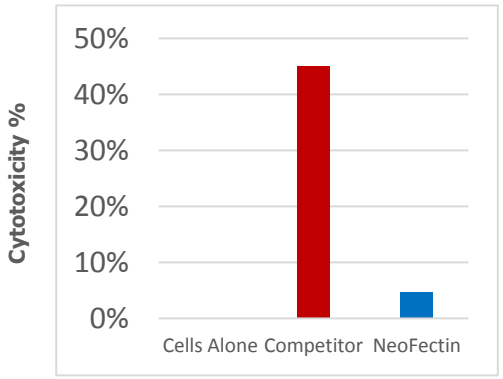
Simple



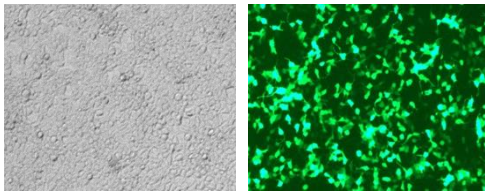
Rapid

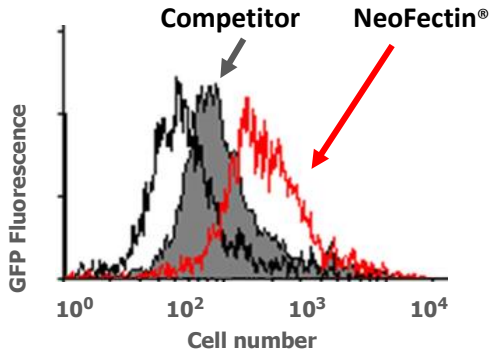


Low Toxicity



High Transfection Efficiency





293 cells, 48 hours post-transfection GFP expression

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RELATED PRODUCTS:
293 Protein Free Medium
CHO Protein Free Medium

Background

Multiple parameters such as the purity of the nucleic acids, the health status of the cells, cell type, and the cell culture medium can effect transfection efficiency. The following protocol should be considered a good starting point. Optimal conditions should be determined by slightly modifying the given conditions until maximum efficiency is achieved. However, the protocol given here should be sufficient to achieve high transfection efficiency with many of the commonly used stable cell lines.

General recommendations for maximum efficiency

Nucleic acids: Highly purified, endotoxin-free and contaminant-free nucleic acids are recommended.

Cell density: We recommend splitting cells 18-24 hours prior to transfection. Cells should be 40-80% confluent at the time of transfection.

Ratio of NeoFectin® to nucleic acid: We recommend using 2µl of NeoFectin® per microgram of nucleic acids. This 2:1 ratio may be varied from 1:1 to 1:4 (µl NeoFectin®: µg nucleic acid) until optimal efficiency is achieved. Recommended starting conditions table provides ratio based on cell culture vessel size.

TRANSFECTION PROTOCOL

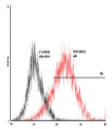
The following protocol is for transfection of adherent cells in a **6-well plate**. For different plate formats follow the recommendations in the Table.

1. 18–24 hours before transfection split cells to achieve 70% confluence at the time of transfection. This equates to plating approximately 4×10^5cells/well. Each well should contain 2ml of complete growth medium.
2. Add 2 µl DNA/or RNA (1 µg/µl) to 94µl complete growth medium.
3. Add 4µl NeoFectin® and vortex gently.
4. Incubate at room temperature 15-20 minutes.
5. Use a pipette to evenly distribute the mixture to the well. There is no need to change medium before adding transfection mixture.
6. Rock the plate to fully distribute the Neofectin®/nucleic acid complexes.
7. Incubate at 37°C, 5% CO2.
8. There is no need to change the medium after adding transfection mixture. If desired medium can be changed 4-6 hours after adding complexes without significant effects on efficiency.
9. Uptake of NeoFectin®/nucleic acid complexes will continue for several hours. If a highly active expression vector is used, expression should be evident within 4-6 hours.
10. Non-adherent cells can also be transfected. We recommend using the protocol described here but starting with 8×10^5 cells in 2ml of complete medium. Scale based on ratios given here.
11. To generate stable cell lines begin selection 24-48 hours after transfection.

Plate cells 18–24 hours before transfection.

Mix nucleic acids and NeoFectin. Incubate 15-20 min

Load the complexes to cells and incubate at 37°C, 5% CO2



Assay 24–72 hours later

Culture vessel	Cells/well	DNA (µg)	NeoFectin® (µl)	Mixture (µl)	Culture Volume (µl)
96-w plate	1×10^4	0.1-0.5	0.2-0.5	10	100
48-w plate	5×10^4	0.2-0.7	0.5-1	20	200
24-w plate	1×10^5	0.5-1	1-2	25	500
12-w plate	2×10^5	1.0-1.5	2-3	50	1000
6-w plate	4×10^5	2-4	4-8	100	2000
35 mm dish	4×10^5	2-4	4-8	100	2000
60 mm dish	1×10^6	5-8	10-15	200	3000
10 cm dish	2×10^6	10-20	20-40	500	5000