Successful Co-Transfection of plasmid-DNA and siRNA using *IsiFect® Transfection Reagent* *

***Data courtesy of Diana Rothe, Ph.D., Postdoctoral Fellow,**
Technische Universität Berlin, Gustav-Meyer-Allee 25, 13355 Berlin, Germany

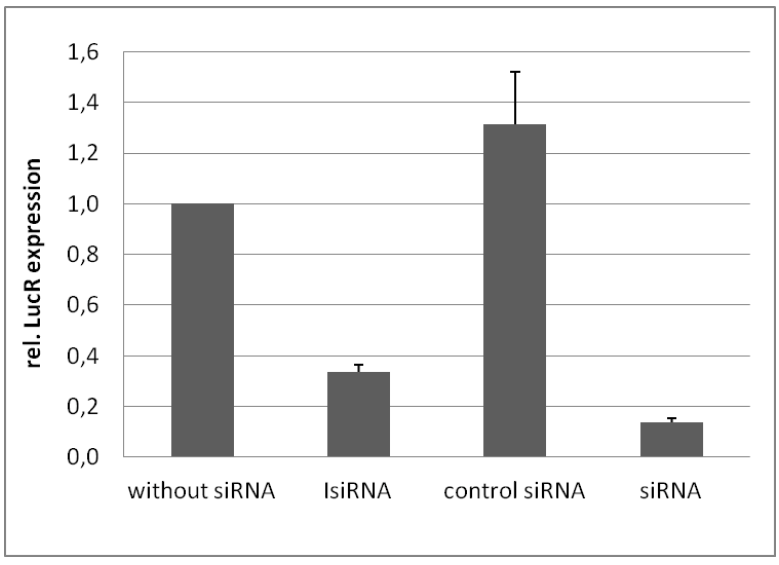
Application Note

The efficient delivery of nucleotides into cells is a crucial step in biotechnology and genetic engineering. Most of the available methods for nucleotide delivery are able to deliver either DNA plasmids or small RNAs into cells. Our aim was to validate a transfection system which is able to encapsulate plasmid-DNA as well as small interfering RNA (siRNA), and deliver both kinds of nucleotides into eukaryotic cell lines in one step. Therefore, we transfected a DNA-Luciferase-reporter plasmid into Neuro2A as well as HEK293 cell line and tried to deliver a siRNA at the same time.

Using IsiFect® Tansfection Reagent, transfection of a DNA-Luciferase-reporter plasmid into Neuro2A cell line as well as into HEK293 cell line was successful indicated by the efficient expression of Luciferase. We also succesfully induced RNAi gene silencing by performing a co-transfection of DNA and siRNA. Furthermore, we observed an efficient delivery of peptide-modified siRNA in order to induce gene silencing.

cell line used: • Neuro2a, HEK293

Gene silencing results (1) using IsiFect® Transfection Reagent



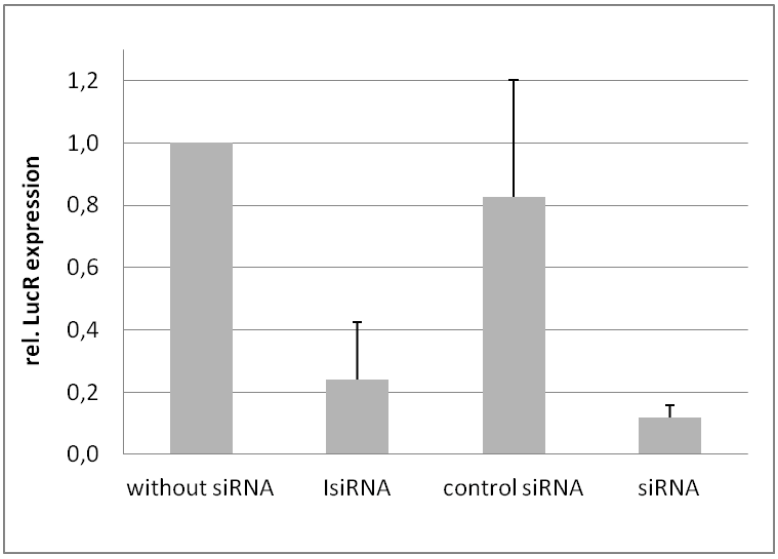
Dual-Luciferase Assay after co-transfection of Neuro2A cell line. Cells were seeded into 24 well plates at a density of 5 x10^4 cells per well. The next day, cells were co-transfected with 0.8 µg of a luciferase reporter plasmid and 100 nM siRNA and IsiRNA, respectively (1,5 µl IsiFect per co-transfection). The used reporter plasmid contains the target sequence of the IsiRNA/siRNA in the 3' UTR of the Renilla luciferase (LucR), and expresses the Firefly luciferase simultaneously for an internal reference.

Optimized Transfection Protocol using IsiFect® Transfection Reagent

- Seed cells into a 24 well plate in a volume of 500 µl per well. The density is dependent on the used cell line.
- Incubate overnight at 37˚C and 5% CO_2. For transfection, cell density should be reached 60-80% confluence.
- Dilute 0,8 µg - 1 µg of a (reporter) plasmid in 100 µl serum-free medium (e.g. RPMI1640).
- Add different volumes of IsiFect, we recommend a range of 1-10 µl.
  Our lab tested 1,5 µl, 3 µl, 5 µl, 10 µl and 15 µl IsiFect®. We observed different cell viabilities 2 days post transfection. Transfection using more than 3 µl IsiFect® showed enhanced toxicity and resulted in more cells that show apoptotic characteristics. Best results were obtained using 1,5 µl IsiFect®.
- Incubate for 15 minutes at room-temperature.
- Add the transfection mixture to the cells and incubate for at least 24 hours.
  Incubation time for commonly used reporter plasmids is around 20 hours.
- Harvest the cells and analyze your samples by a method of choice.

Application Note

Gene silencing results (2) using IsiFect® Transfection Reagent



Dual-Luciferase Assay after co-transfection of HEK293 cell line. Cells were seeded into 24 well plates at a density of 5 x10^4 cells per well. The next day, cells were co-transfected with 0,8 µg of a luciferase reporter plasmid and 100 nM siRNA and IsiRNA, respectively (1,5 µl IsiFect per co-transfection). The used reporter plasmid contains the target sequence of the IsiRNA/siRNA in the 3' UTR of the Renilla luciferase (LucR), and expresses the Firefly luciferase simultaneously for an internal reference.

Conclusion

IsiFect® Transfection Reagent efficiently delivers siRNA and DNA-plasmids into cell lines in co-transfection experiments.