FuGENE® SI – A novel and efficient siRNA transfection reagent with minimal cellular toxicity

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Introduction

The use of siRNAs and miRNAs to modulate gene expression is a powerful tool in functional genomics research and is increasingly being adopted into clinical therapeutic applications. As such, there is a need for next-generation delivery systems which can safely and efficiently deliver RNA into a wide range of eukaryotic cells and tissues. We present to you, FuGENE SI®, a novel, and highly efficient siRNA Transfection Reagent that is extremely gentle on cells. Utilizing our proprietary chemical and lipid technology, FuGENE SI®becomes FuGENE's first transfection reagent engineered and optimized for siRNA and miRNA delivery.

FuGENE® SI provides researchers the following benefits when compared to other siRNA transfection reagents available on the market:

- Improved gene silencing with low siRNA amounts
- Decreased cellular toxicity
- Usage across a wide range of routine and difficult-to-transfect cell lines
- Quick and easy protocol
- Ability to co-transfect DNA
- Lower Costs

To demonstrate the utility and benefits of utilizing FuGENE® SI we present to you the following:

- Flexible and Rapid Transfection Protocol with FuGENE®SI: Allows researchers to seed cells and transfect the same day, perfect for high-throughput screening (HTS) applications
- FuGENE® SI Transfection reagent allows for highly efficient uptake of fluorescently labeled siRNAs
- Robust and gentle knockdown of overexpressed, green florescent protein in eukaryotic cell lines with minimal amounts of siRNAs delivered by FuGENE®SI
- Improved knockdown performance of FuGENE®SI vs. competitor reagent RNAiMax®in human breast cancer cell line
- Knockdown of endogenous FXYD1 in A549 with FuGENE® SI increases cell survival after treatment with antibody/drug conjugate (FXYD1/Cardiac Glycoside)

Flexible and Rapid Transfection Protocol

FuGENE® SI was engineered to allow for quick and easy set-up of transfection reactions, and provides researchers flexibility in selecting a protocol based on downstream applications. Choose our traditional forward protocol if you'd like to prepare your cells the day before, or our rapid protocol if you'd like to prepare cells and transfect on the same day. For high throughput screening applications, you can also use our fast and easy reverse-transfection protocol where cells are directly added to the microplate containing the FuGENE® SI and siRNA complex

Traditional/Forward Transfection Protocol

- Day 0: One day before transfection, adjust cell concentration and seed cells in culture vessel according to users guide.
- Day 1: Form siRNA/FuGENE®complex by incubating diluted siRNA and FuGENE® SI in DMEM for at least 5 minutes. Then add complex to cells, swirl to mix, and incubate for 24-72 hours
- Day 2-4: Analyze cells for gene/protein knockdown via chosen method

Rapid/Forward Transfection Protocol

- Day 1: On the day of transfection, seed cells in culture vessel according to users guide. (For rapid transfection use 2x the amount of cells you would typically seed for a traditional forward transfection). Form siRNA/FuGENE®complex by incubating diluted siRNA and FuGENE® SI in DMEM for at least 5 minutes. Then add complex to cells, swirl to mix, and incubate for 24-72 hours
- Day 2-4: Analyze cells for gene/protein knockdown via chosen method

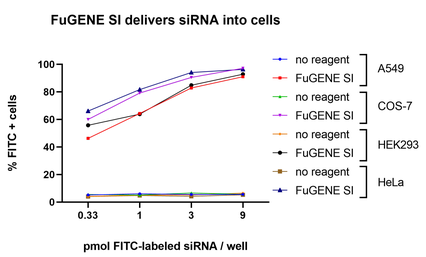
Reverse Transfection/HTS Protocol

- Day 1: On the day of transfection, prepare siRNA/FuGENE® SI complex by incubating diluted siRNA and FuGENE® SI in DMEM in assay plate or vessel for at least 5 minutes. Add cells directly to the wells containing the siRNA/FuGENE® SI complex, swirl to mix, and incubate for 24-72 hours.
- Day 2-4: Analyze cells for gene/protein knockdown via chosen method

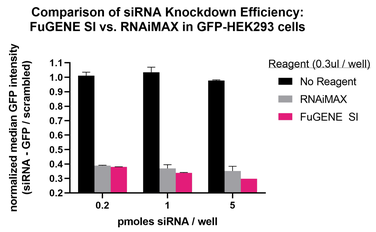
Highly efficient uptake of FITC-labeled small RNAs

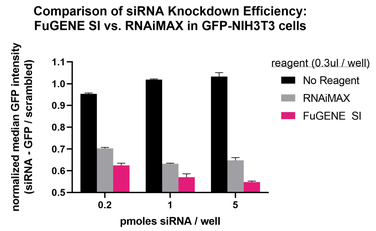
A549, COS7, HEK293 and HeLa cells plated in 96-well microplates were transfected with 0.4ul of FuGENE SI or negative control no reagent and various amounts of FITC-labeled negative control siRNA. 24 hours after transfection the cells were analyzed via flow cytometry for FITC-positive signal.

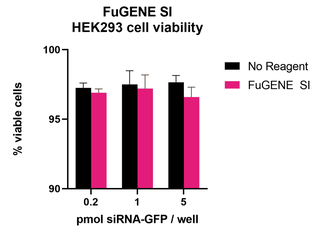
- FuGENE® SI allows for highly efficient uptake of labeled small RNAs

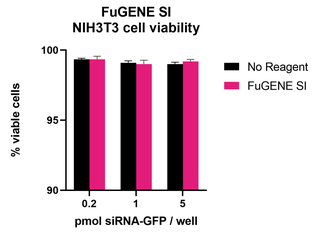


Highly effective gene knockdown of overexpressed protein with minimal siRNA amounts.





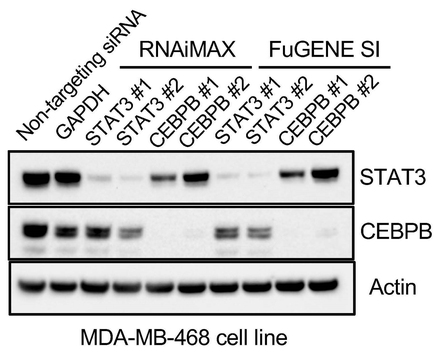




- Stable expressing HEK293-GFP & NIH3T3-GFP Cell lines were seeded in 96-well plates according to user guides and subsequently transfected with 0.2, 1.0, or 5 pmols of GFP targeting siRNA or negative control (Horizon Discovery/Dharmacon®), along with 0.3ul of Transfection Reagent FuGENE® SI or 0.3ul of Lipofectamine® RNAiMax (Life Technologies Corporation). Cells were then analyzed via flow cytometry 48 hours post-transfection to measure % knockdown of GFP & total cell viability. Graphs above show the superior knockdown performance of FuGENE® SI vs. Lipofectamine® RNAiMax in HEK293 and NIH3T3 cells.

- FuGENE® SI is highly effective in delivering low amounts of siRNAs to knockdown overexpressed proteins

Improved knockdown performance of FuGENE® SI compared to competitor transfection reagent RNAiMax®

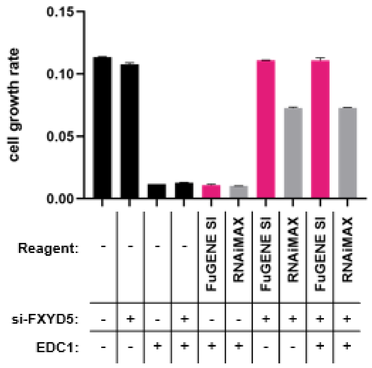


- Human MDA-MB-468 cells were reverse transfected in 6-well plates with 40pmol of the indicated targeting siRNAs or negative/positive control (Silencer Select®Life Technologies) utilizing either 7.5ul FuGENE® SI Transfection Reagent or 7.5ul RNAiMax®per well. 72 hours after transfection, the cells were collected and analyzed via western blot for expression of target genes STAT3 & CEBPB. (Dr. Dai Horiuchi, Northwestern University)

- FuGENE® SI enables robust knockdown of STAT3 and CEBPB in MDA-MB-468 Breast Cancer cell line as confirmed by western blotting

- FuGENE®SI yielded improved knockdown performance as compared to market leading transfection reagent RNAiMax® (Life Technologies Corp.)

Knockdown of FXDY5 in human A549 increases cell survival without growth impairment after treatment with FXYD5-antibody/Cardiac-Glycoside conjugate

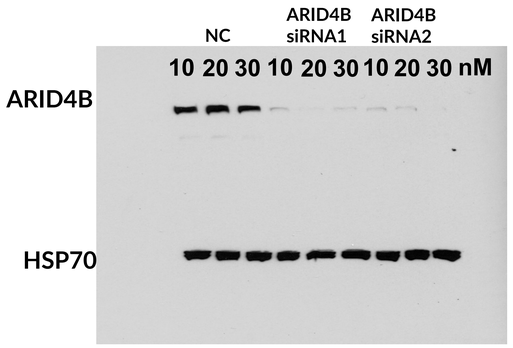


- Human A549 cells plated in 96-well microplates were transfected with +/- 1 pmol of FXYD5-targeting siRNA (Dharmacon®siGENOME, Smart Pool), utilizing either 0.3ul FuGENE® SI Transfection Reagent or 0.3ul of RNAiMAX®. 24 hours after transfection, the cells were treated with +/- 1 nM EDC1 (an extracellular FXYD5 antibody-cardiac glycoside conjugate that binds to and kills FXYD5-expressing cells). The cell growth rate was then measured by the generation of lactic acid via microplate reader absorbance detection.

- **FuGENE® SI yields highly efficient knockdown of endogenously expressed FXYD5**
- **FuGENE®SI is non-toxic and does cause impairments to cellular growth**

Robust knockdown of endogenous ARID4B in MCF-7

- Human MCF-7 cells plated in 6-well microplates were transfected with 10, 20 or 30 nM of ARID4B targeting siRNAs or negative control (Millipore Sigma, Mission®siRNAs) utilizing 7.5ul FuGENE® SI Transfection Reagent per well. 48 hours after transfection, the cells were collected and 20ug of total cell lysate was analyzed via western blot for expression of target gene ARID4B and control gene HSP70. (Dr. Wu Lab, George Washington University)



- FuGENE® SI enables robust knockdown of endogenously expressed ARID4B in human MCF-7 breast cancer cell line as confirmed by western blot, and does not cause unintended off-target knockdown of control loci.

Conclusions

FuGENE® SI is a novel, high efficiency & non-toxic transfection reagent for the delivery of siRNA and short RNAs into biologically relevant eukaryotic cell lines. Usage of FuGENE®SI enables researchers to experience the following benefits:

- Robust and improved gene silencing with low siRNA amounts
- Decreased cellular toxicity with no impairments to cellular growth
- Usage across a wide range of routine and difficult-to-transfect cell lines
- Quick and easy protocol
- Ability to co-transfect DNA
- Lower Costs