SilenceMag Results

OZ Biosciences is pleased to announce the launching of a new Magnetofection ™ - based product, specifically designed for siRNA application: **SilenceMag**. Magnetofection™ is a new revolutionary nucleic acids delivery method. It exploits magnetic force to drive the nucleic acids vectors associated with magnetic particles towards, possibly even into, the target cells. In this manner, the complete applied dose of siRNA gets concentrated on the cells within a few minutes so that 100% of the cells get in contact with a significant dose of siRNA. In this way, **SilenceMag** is the most efficient and powerful siRNA delivery systems available. Its efficacy allows studying Gene Silencing at very low dose of siRNA (< 10 nM) saving materials and time. **SilenceMag** formulation gives reliable higher Gene silencing efficiencies in numerous cell types than any other transfection reagent.

Main **SilenceMag** features are:

1. Greatly improved the percentage of cells transfected compared to standard methods.
2. Use 10 to 100 times less siRNA and achieve high gene silencing.
3. One product for all siRNA application: Suitable for co-transfection & endogenous gene silencing
4. Extremely short process time in comparison to standard procedures.
5. Serum compatible, Fast and easy, Non-toxic

OZ Biosciences offers three types of ready-to-use reagents: **PolyMag** suitable for all nucleic acids, **CombiMag** designed to be associated with all vectors: transfection reagents and viruses and **SilenceMag**. **SilenceMag** has been designed specifically and used successfully to deliver siRNA, shRNA and dsRNA. **SilenceMag** is a universally applicable magnetic particle preparation for high transfection efficiency. siRNA to be transfected and the magnetic particles are mixed in a one-step procedure.

Nucleic Acid Types

Nucleic Acid or Virus Type	PolyMag	SilenceMag	CombiMag
DNA (plasmid)	√	NA	√
Oligonucleotides	√	ND	√
mRNA	√	ND	√
siRNA	√	√	√
dsRNA	ND	√	√
shRNA	ND	√	√
Adenovirus	NA	NA	√
Retrovirus	NA	NA	√

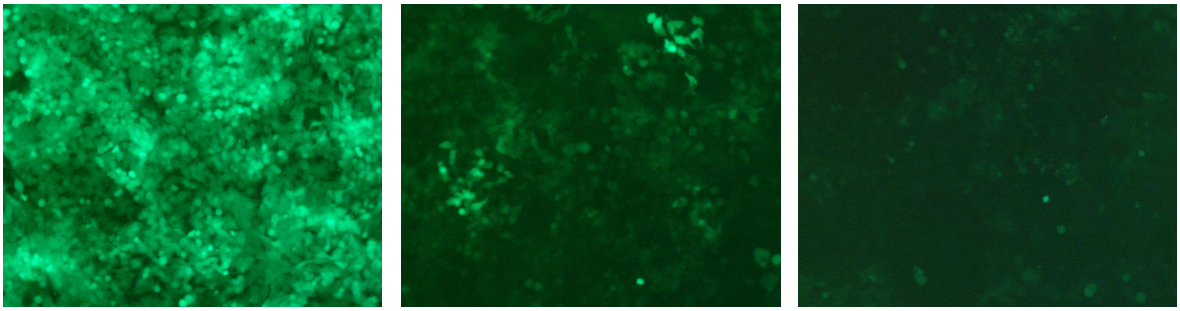
Cell Types

SilenceMag is applicable and has been tested successfully on a variety of immortalized cell lines (HeLa, COS7, N2A, 293-HEK, MDCK, NIH3T3...) and primary cells (HUVEC). Please consult our updated list of cells successfully tested available on the website: www.ozbiosciences.com. If a particular cell type or cell line is not listed, this does not imply that **SilenceMag** is not going to work. OZ Biosciences is going to frequently adjust this list.

Targeted Genes

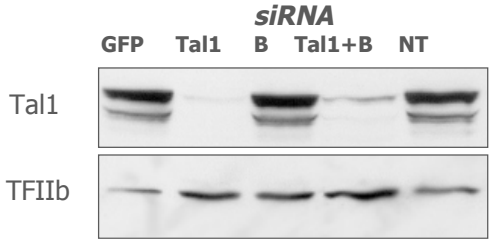
siRNA gene silencing mediated by **SilenceMag** has been tested successfully towards various targets. Gene silencing efficiency has been demonstrated in co-transfection (targeted genes: GFP and Lac Z), in stably transfected cells (targeted genes: GFP and Luciferase) and with endogenous target such as GAPDH, Lamin and transcription factors. Please consult our updated results available on the website: www.ozbiosciences.com.

High Gene Silencing Efficiency Mediated by SilenceMag / siRNA Complexes



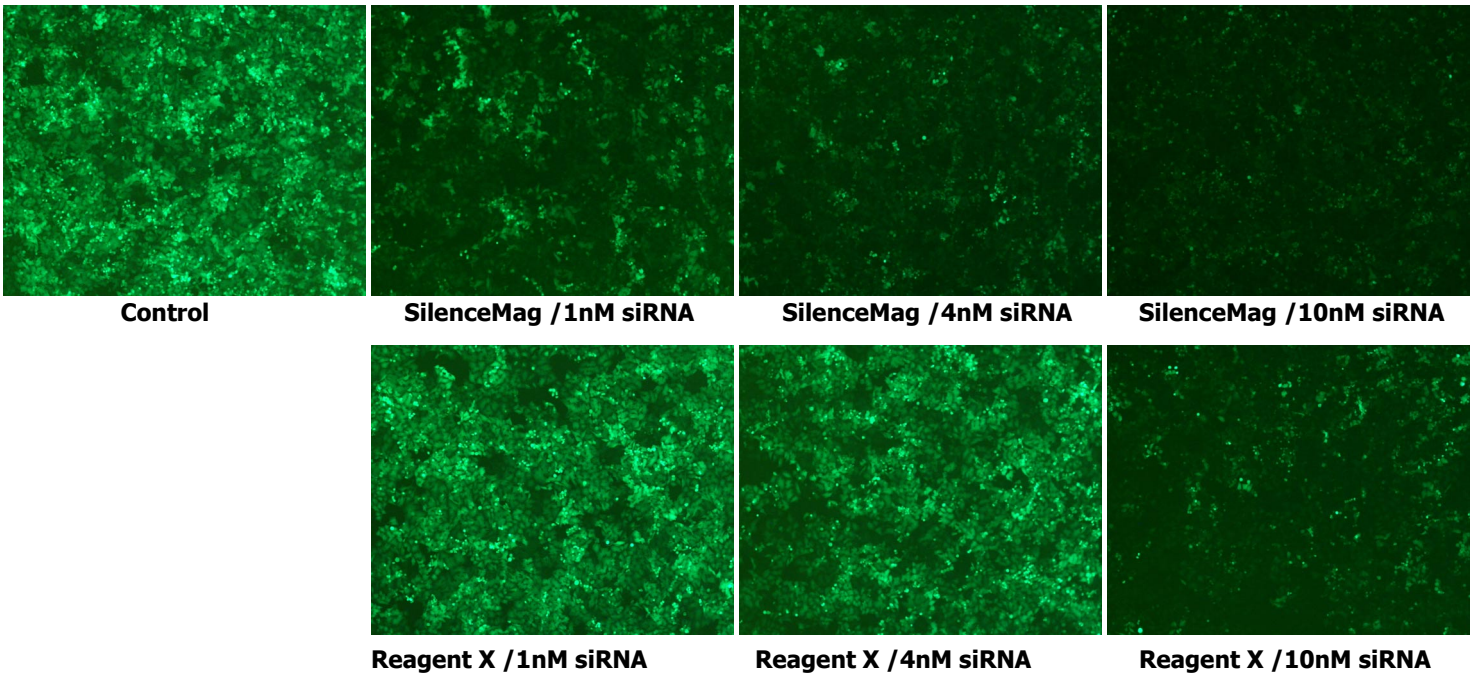
Control **SilenceMag /4nM siRNA** **SilenceMag /10nM siRNA**

HeLa Cells stably transfected with GFP were treated with **SilenceMag** and siRNA (targeting GFP) as described in the instruction manual. Complexes were prepared with 0.5 µl of **SilenceMag** and 4nM (10.8 ng) or 10nM (27ng) siRNA. Cells seeded in 96-well plate were then transfected in 200µl transfection volume. GFP expression was monitored 72 h post-transfection by fluorescence microscopy.



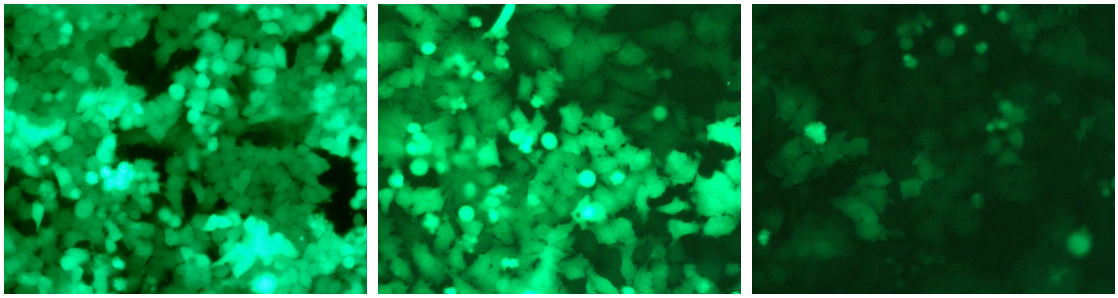
Primary Human Umbilical Vein Endothelial Cells (HUVEC). *Target siRNA*: Transcription factors Tal1 - *Control siRNA*: Transcription factors B (Belongs to Tal-1 family) and GFP - *NT*: Non-transfected cells - *Cell culture dish*: $3x10^5$ cells / 60 mm dish - *siRNA final concentration:* 25nM. **SilenceMag** and siRNA were prepared in serum free medium, incubated on cells 15 minutes with the magnetic plate, culture medium was then washed and new serum containing medium was added to the cells. Cell lysates were prepared 24 hours after siRNA treatment and analyzed by western blot with an anti-TAL 1 antibody, re-hybridized with anti- TFIIB (ubiquitous transcription factor) antibody as control.
We are grateful to Dr. D. Matthieu (CNRS-UMR5535, Montpellier) for kindly providing these data.

siRNA Dose Response in HeLa cells: SilenceMag vs Reagents X & Y



Control **SilenceMag /1nM siRNA** **SilenceMag /4nM siRNA** **SilenceMag /10nM siRNA**

Reagent X /1nM siRNA **Reagent X /4nM siRNA** **Reagent X /10nM siRNA**

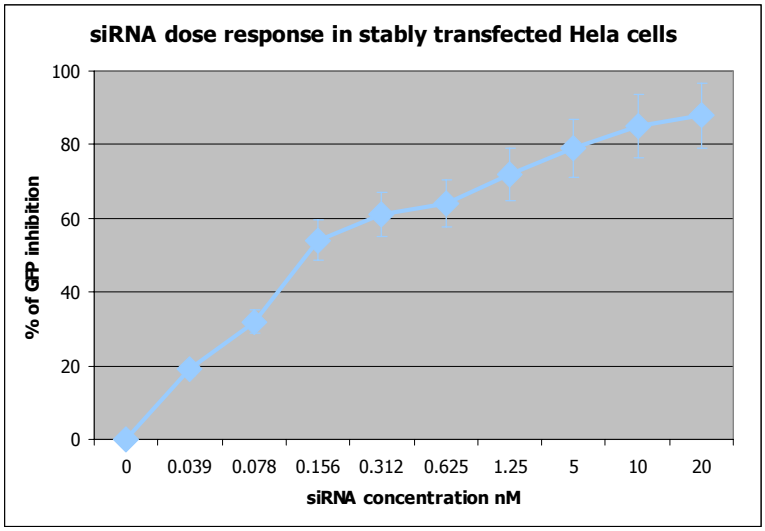


Control **Reagent Y /5nM siRNA** **SilenceMag /5nM siRNA**

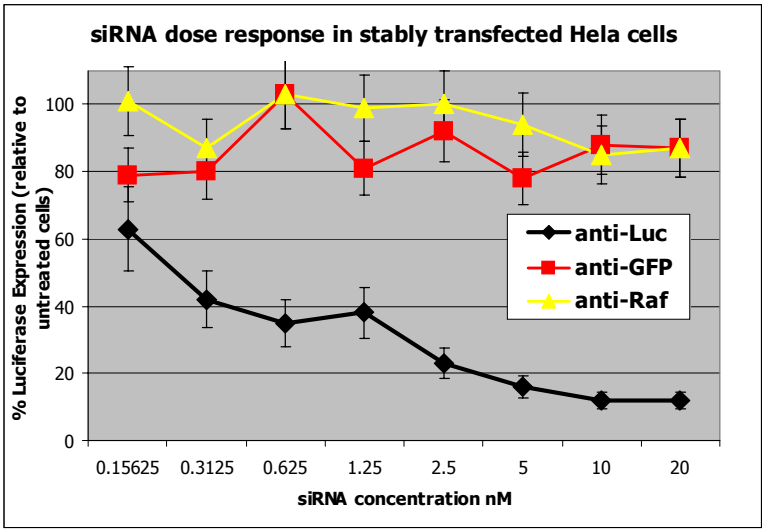
GFP-stably transfected **HeLa Cells** were assayed with **SilenceMag** and siRNA (targeting GFP) as described in the instruction manual. Complexes were prepared with 0.5 µl of **SilenceMag** and 1nM, 4nM, 5nM or 10nM siRNA. Cells seeded in 96-well plate were then transfected in 200µl transfection volume. GFP expression was monitored 72 h post-transfection by fluorescence microscopy. Commercial siRNA transfection reagents X and Y were used according manufacturer's protocol.

siRNA Dose Response in HeLa- GFP and HeLa –Luciferase Cells

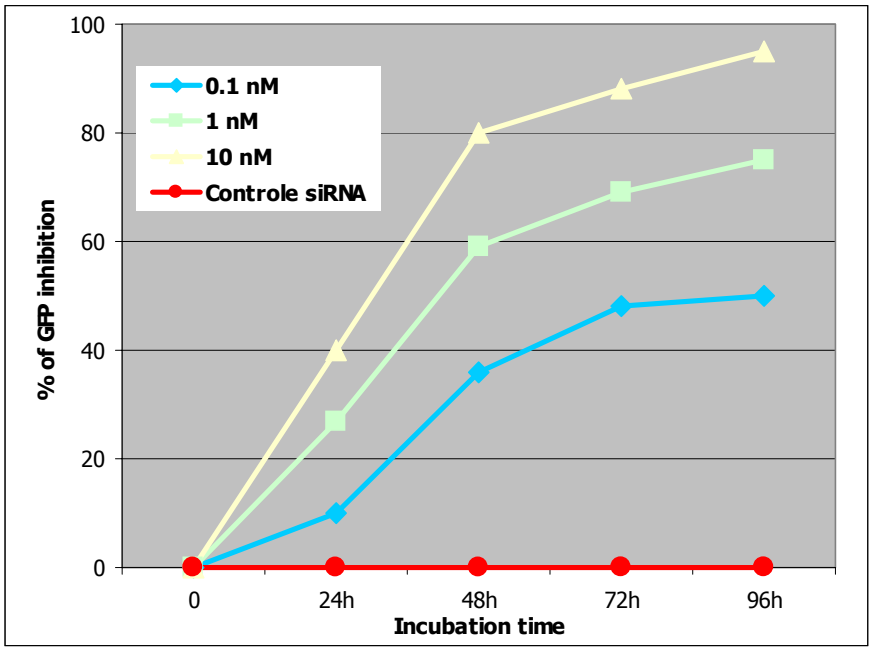
GFP-stably transfected **HeLa Cells** were assayed with **SilenceMag** and siRNA (targeting GFP) as described in the instruction manual. Cells seeded in 96-well plate were then transfected in 200µl transfection volume. GFP expression was monitored 72 h post-transfection. Results show percentage of GFP inhibition.



Luciferase-stably transfected **HeLa Cells** were assayed with **SilenceMag** and siRNA as described in the instruction manual. Cells seeded in 96-well plate were then transfected in 200µl transfection volume. Luciferase expression was monitored 48 h post-transfection. Results show percentage of luciferase expression.

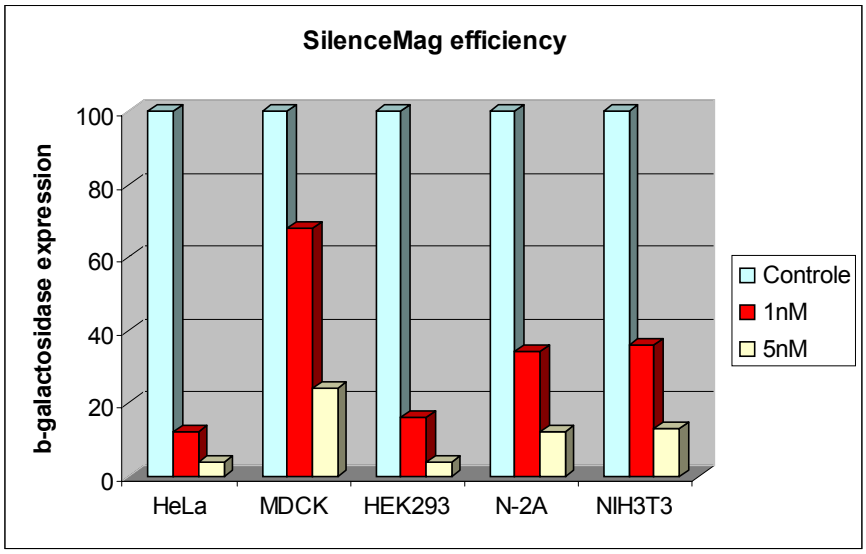


Gene Silencing Time Course



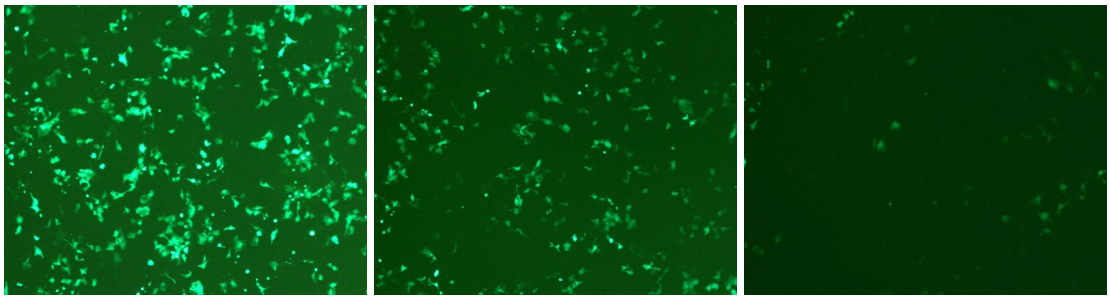
GFP-stably transfected **NIH-3T3 Cells** were assayed with **SilenceMag** and siRNA as described in the instruction manual. Three siRNA concentrations were monitored 0.1nM, 1nM & 10nM. Cells seeded in 96-well plate were then transfected in 200µl transfection volume. GFP expression was recorded in function of post-transfection incubation time.

SilenceMag Efficiency in Various Cells Co-Transfected with Lac Z Gene



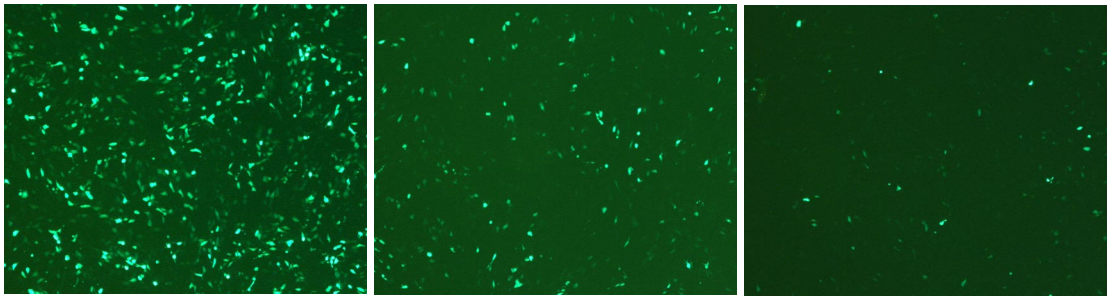
Various cells (HeLa, MDCK, KEK-293, N2A and NIH-3T3) were co-transfected in 96-well plates with 100 ng of pLacZ plasmid complexed to 0.1 µl of **PolyMag** reagent and with siRNA associated with **SilenceMag**. β-Galactosidase expression was monitored 48 h after transfection using OZ Biosciences' β-Galactosidase assay kit (catalog number GO-10001).

NIH-3T3



Control **SilenceMag/ 5nM siRNA** **SilenceMag/ 10nM siRNA**

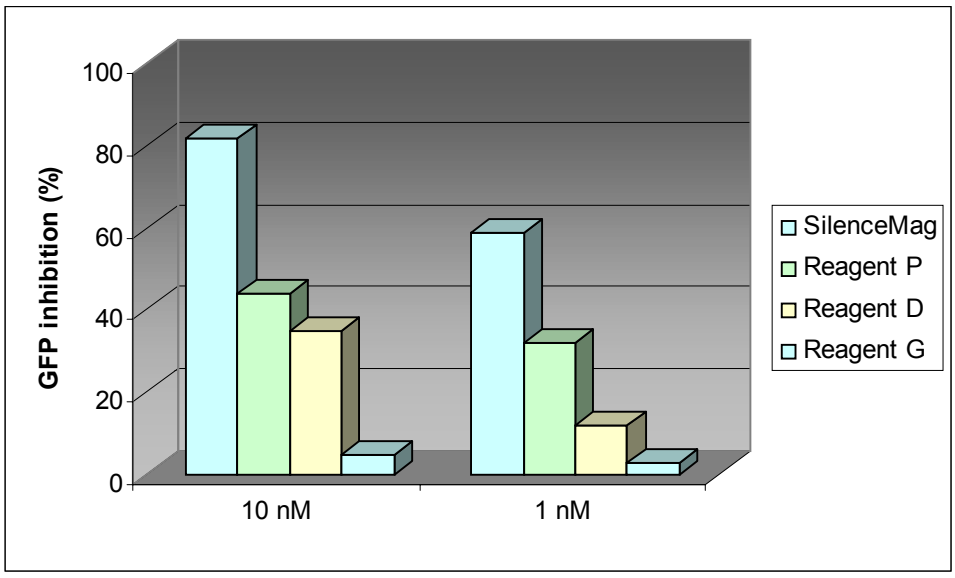
COS-7



Control **SilenceMag/ 1nM siRNA** **SilenceMag/ 10nM siRNA**

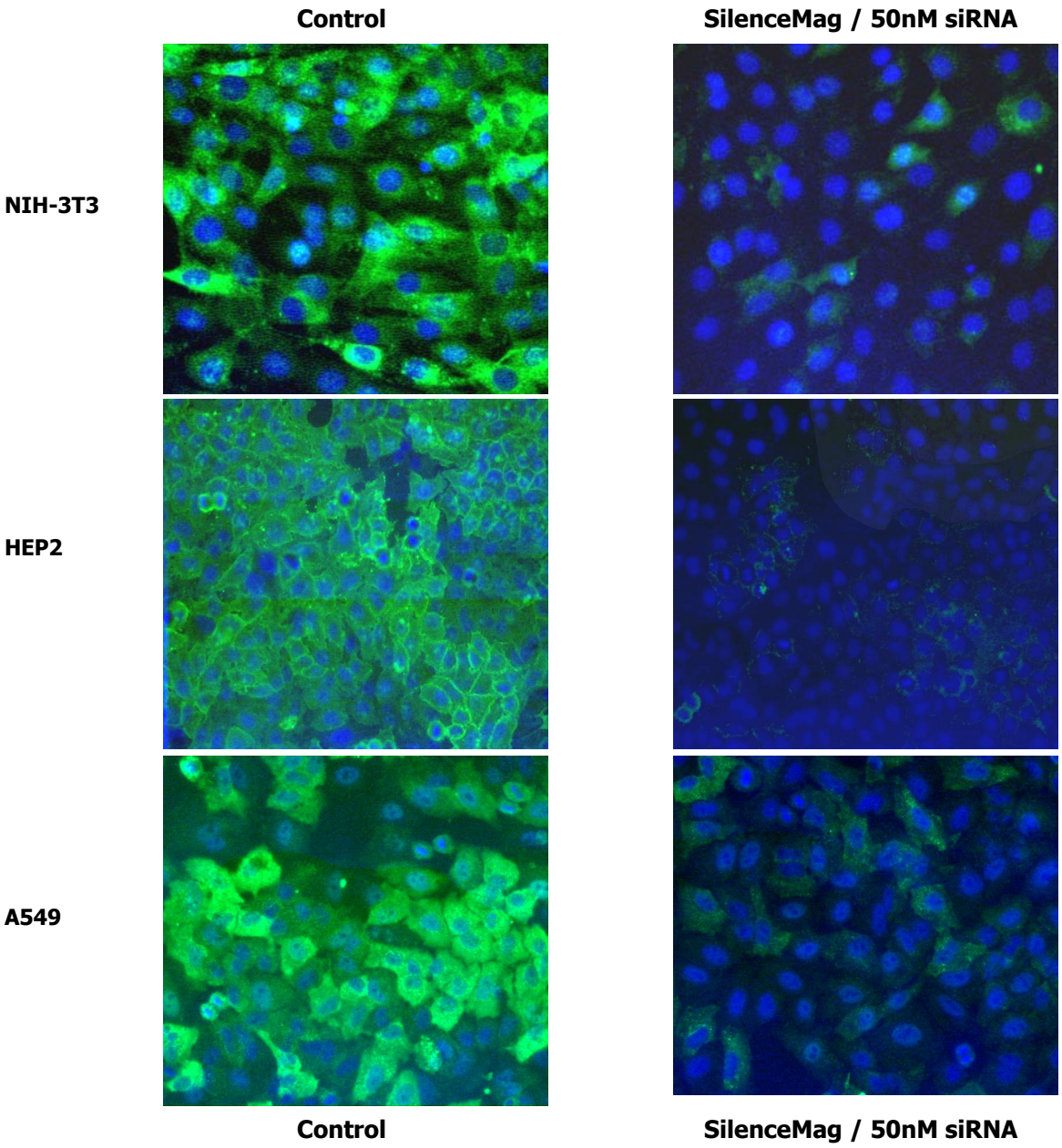
NIH-3T3 and COS7 cells were co-transfected in 24-well plates with 100 ng of pGFP plasmid complexed to 0.1 µl of **PolyMag** reagent and with siRNA associated with **SilenceMag**. GFP expression was monitored 48 h after transfection.

Comparison of SilenceMag Gene Silencing Efficiency with Other siRNA Reagents



GFP-stably transfected **HeLa Cells** were assayed with **SilenceMag** and siRNA (targeting GFP) as described in the instruction manual. Cells were transfected in 24-well plate. GFP expression was monitored 48 to 72 h post-transfection. Reagent P, D & G, commercial siRNA transfection reagents were used according manufacturer's protocol.

SilenceMag Efficiency to Target Endogenous Gene in NIH3T3: GAPDH



NIH-3T3 (top), Hep2 (middle) and A549 (bottom) cells were treated with **SilenceMag** and siRNA (targeting GAPDH gene). Complexes of **SilenceMag** and siRNA were performed as described in the instruction manual – [5 µl of **SilenceMag** and 50nM (335 ng) siRNA]. Cells were transfected in 24-well plate with a transfection volume of 0.5mL. GAPDH expression was monitored 48 to 72 h post-transfection by immunocytochemistry. FITC-anti-GAPDH antibody and DAPI (label nucleus in blue) were detected by fluorescent microscopy

Bibliographic References

Please consult our list of references available on the website: www.ozbiosciences.com.