Red FluoMag Results

Magnetofection™ is a simple and highly efficient method to transfect cells in culture and in vivo. OZ Biosciences offers four ready-to-use reagents based on this method:
- **PolyMag** designed for all DNA transfection and all nucleic acids delivery (RNA, ODN, siRNA…)
- **SilenceMag** for siRNA applications
- **CombiMag** for enhancing all transfection reagents efficiency
- **ViroMag** for viral transductions

Red FluoMag reagents are tetramethylrhodamine-conjugated magnetic nanoparticles. These red fluorescent reagents are useful for many applications. For instance:
- Double labeling and co-localization studies, with GFP or FITC labeled nucleic acids
- FACS analysis, fluorescent and confocal microscopy
- Transfection mechanisms follow (interaction with cells, intracellular pathway…)
- Fluorescence resonance energy transfer (FRET) assay as well as tracking internalization pathway in endocytic vesicles.
- Determine complexes stability in various biological environment
- Analyze the association of nucleic acids or transfection reagents or viruses with the magnetic nanoparticles

Four different fluorescently-labeled magnetic nanoparticles are available:
- **FluoMag-P** corresponding to *PolyMag* (DNA transfection and nucleic acids delivery)
- **FluoMag-S** analogous to *SilenceMag* (siRNA applications)
- **FluoMag-C** corresponding to *CombiMag* (for enhancing all transfection reagents efficiency)
- **FluoMag-V** equivalent to *ViroMag* (viral applications)

Nucleic Acid Types

Nucleic Acid or Virus Type	FluoMag-P	FluoMag-S	FluoMag-C	FluoMag-V
DNA (plasmid)	√	NA	√	NA
Oligonucleotides	√	ND	√	NA
mRNA	√	ND	√	NA
siRNA	√	√	√	NA
dsRNA, shRNA	√	√	√	NA
Viruses	NA	NA	√	√

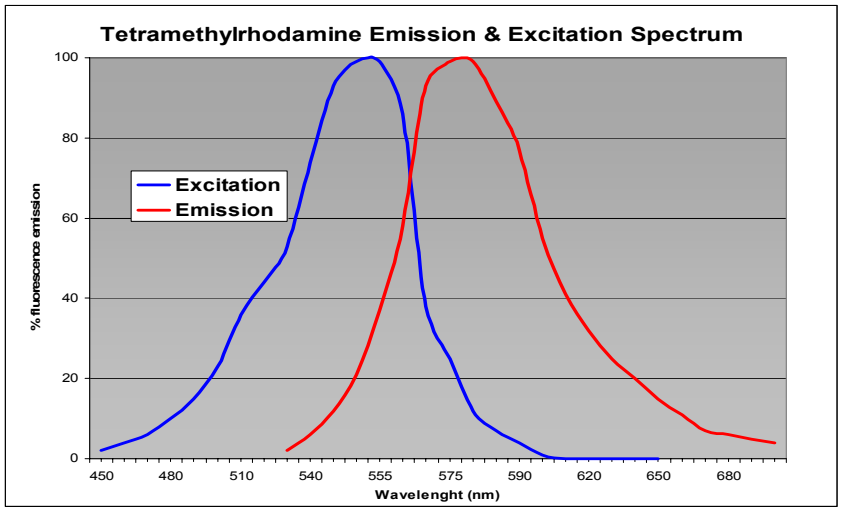
NA: not applicable; ND: not determined

Cell Types

FluoMag reagents are usually applicable on many cell types. This technology has been tested successfully on a variety of immortalized and primary cells. 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 **FluoMag** reagents are not going to work.

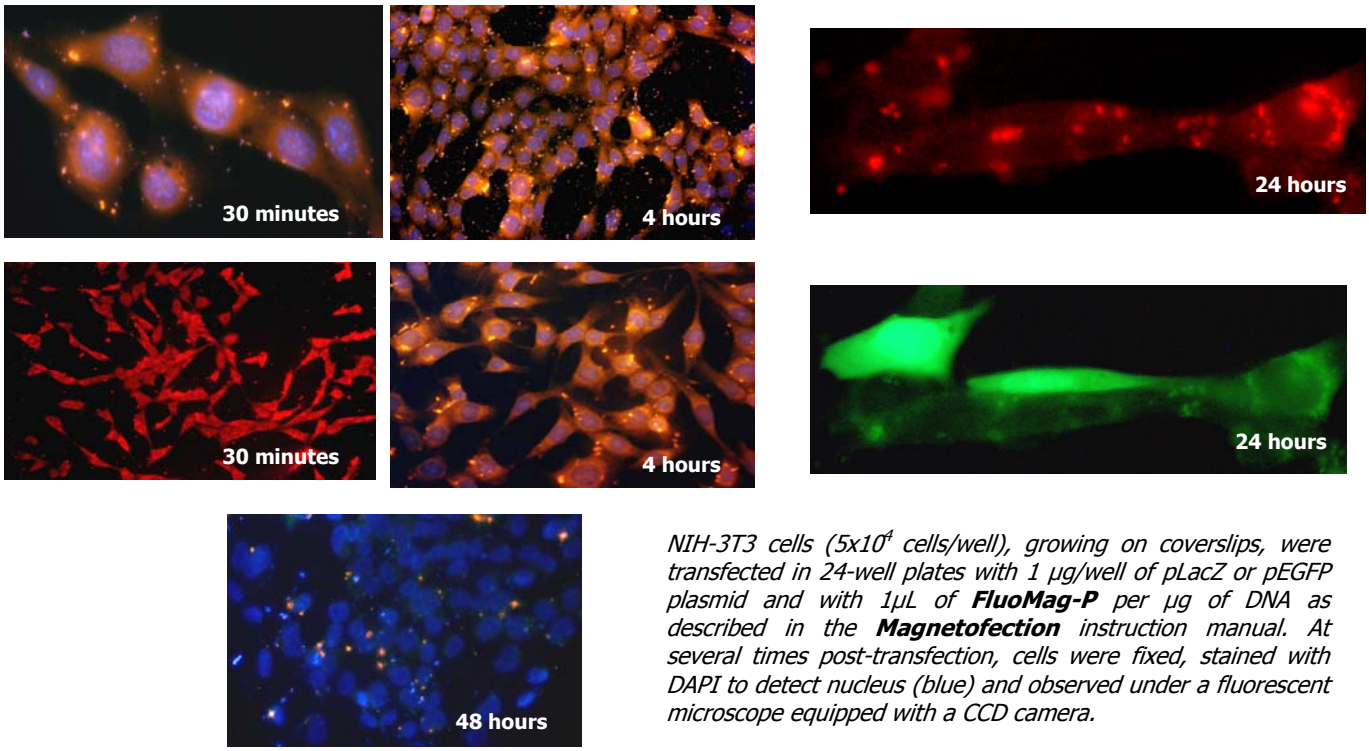
Tetramethylrhodamine Probe

The **FluoMag** reagents are labeled with a rhodamine fluorophores (red) that is visualized in the visible spectrum. The excitation peak is at 555 nm and the emission maximum at 580 nm. It is ideal for FACS, fluorescent and confocal microscopy and most fluorescent detection system. This red probe is pH insensitive and therefore suitable for fluorescence resonance energy transfer (FRET) studies. The label is covalently coupled to the magnetic nanoparticles and cannot leave the nanoparticles upon nucleic acid interactions or internalization.



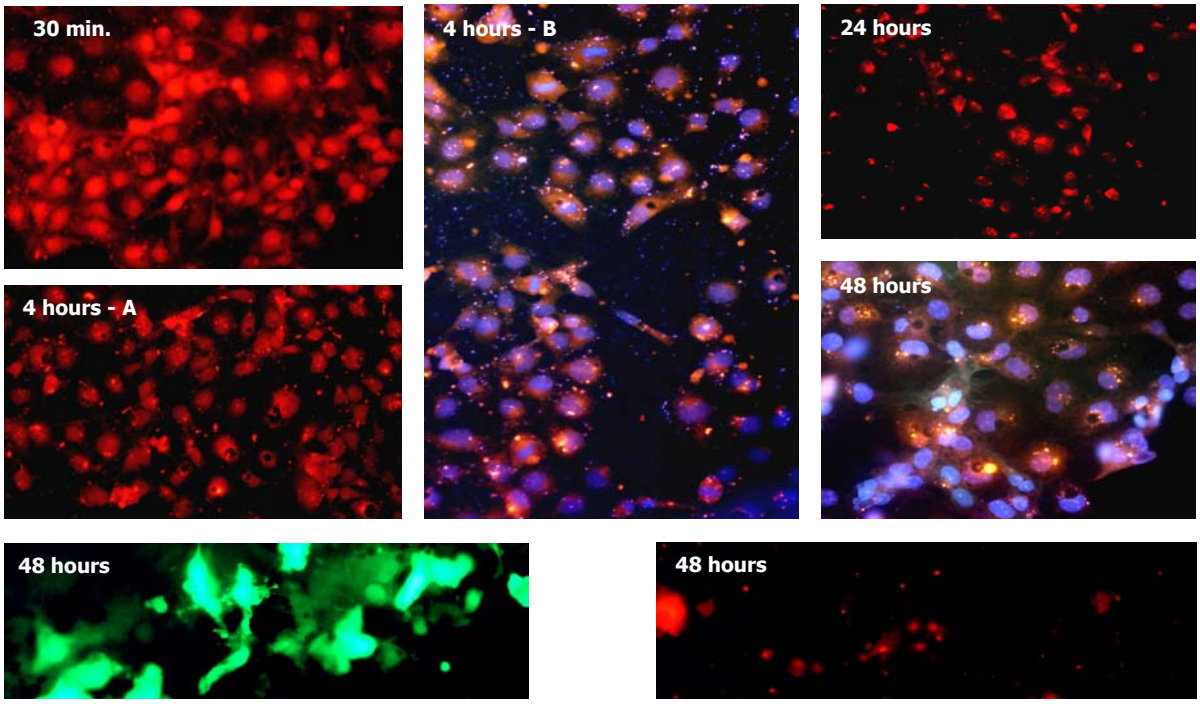
Fluorescent probe	Max Excitation Wavelength (nm)	Max Emission Wavelength (nm)	pH Sensitive	ε (Cm^{-1} M^{-1})	CF 280 (A_{280} free dye / A_{max} free dye)	Laser range
TRITC	555	580	no	65,000	0.30	visible

Transfection of NIH-3T3 cells with plasmid DNA and FluoMag-P



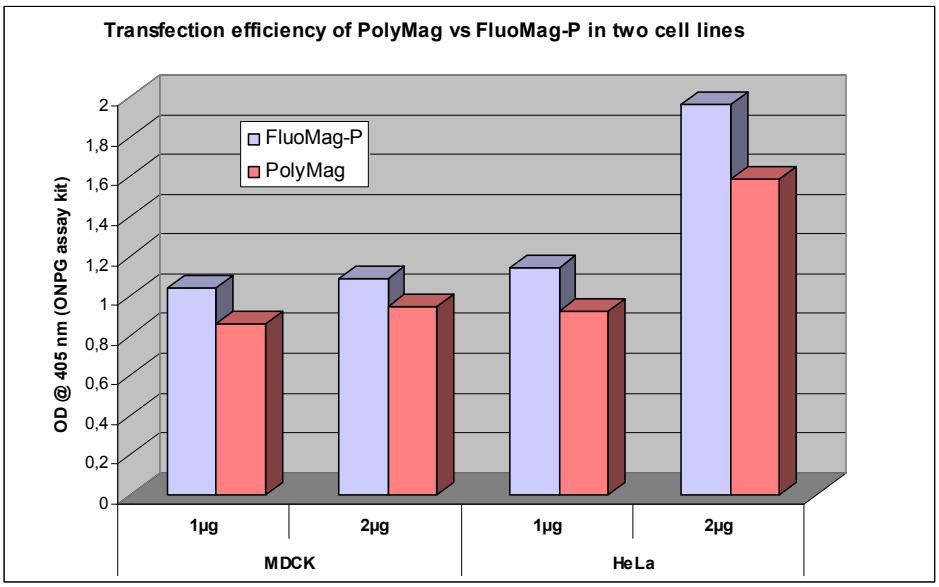
*NIH-3T3 cells (5x10^4 cells/well), growing on coverslips, were transfected in 24-well plates with 1 µg/well of pLacZ or pEGFP plasmid and with 1µL of **FluoMag-P** per µg of DNA as described in the **Magnetofection** instruction manual. At several times post-transfection, cells were fixed, stained with DAPI to detect nucleus (blue) and observed under a fluorescent microscope equipped with a CCD camera.*

Transfection of COS 7 cells with plasmid DNA and FluoMag-P



*COS7 cells (5x10^4 cells/well), growing on coverslips, were transfected in 24-well plates with 1 µg/well of pLacZ or pEGFP plasmid and with 1µL of **FluoMag-P** per µg of DNA as described in the **Magnetofection** instruction manual. At several times post-transfection, cells were fixed and observed under a fluorescent microscope equipped with a CCD camera. At 30 minutes and 4 hours-A post transfection, only rhodamine fluorescence was analyzed. In some pictures, nuclei were stained with DAPI (blue).*

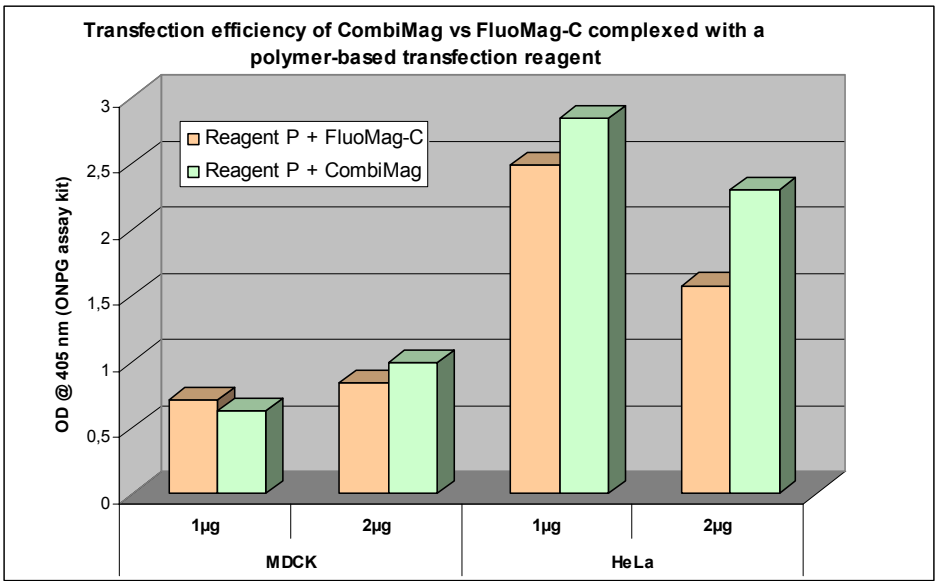
Transfection Efficiency of FluoMag-P versus PolyMag



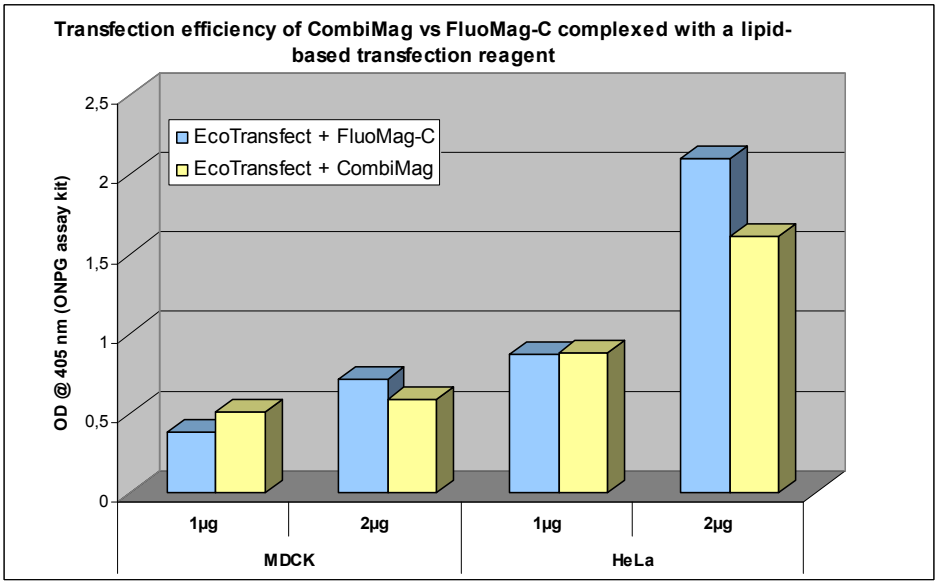
*HeLa and MDCK cells (5x10^4 cells/well) were transfected in 24-well plates with 1 or 2 µg/well of pLacZ plasmid and with 1µL of **PolyMag** or **FluoMag-P** per µg of DNA as described in the **Magnetofection** instruction manual. β-Galactosidase expression was revealed 48 hours after transfection using **OZ Biosciences' ONPG assay kit** (catalog # GO10001).*

Conclusions: The **PolyMag** reagent and its fluorescently labeled counterpart, **FluoMag-P** have comparable transfection efficiency. Thus, the labeling procedure did not affect the activity of the magnetic nanoparticles for transfecting DNA.

Transfection Efficiency of FluoMag-C versus CombiMag



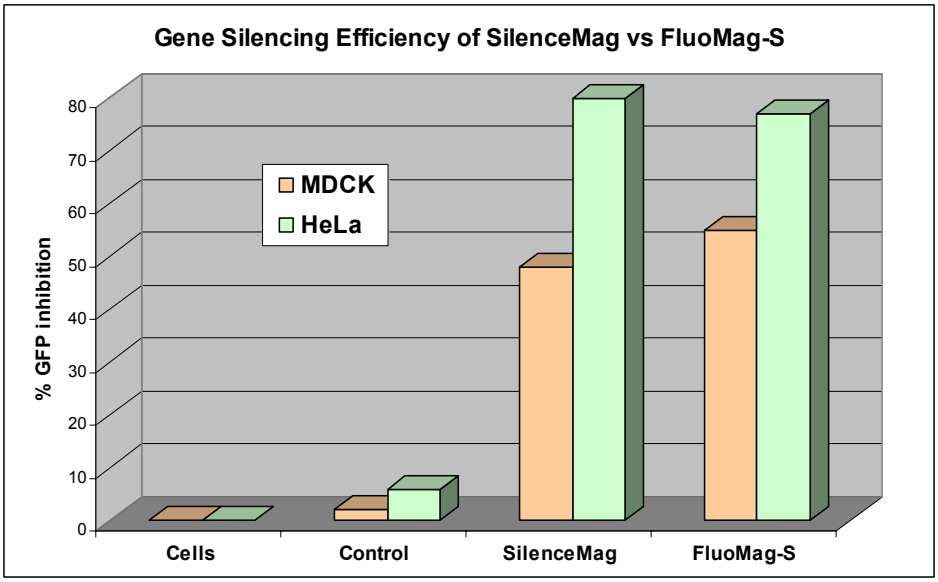
*HeLa and MDCK cells (5x10^4 cells/well) were transfected in 24-well plates with 1 or 2 µg/well of pLacZ plasmid and a polymer-based transfection reagent (reagent P) complexed to either **CombiMag** or **FluoMag-C**, according to the **Magnetofection** instruction manual and the transfection reagent P manufacturer's instruction. β-Galactosidase expression was revealed 48 hours after transfection using **OZ Biosciences' ONPG assay kit***



*HeLa and MDCK cells (5x10^4 cells/well) were transfected in 24-well plates with 1 or 2 µg/well of pLacZ plasmid and a lipid-based transfection reagent (**EcoTransfect**) complexed to either **CombiMag** or **FluoMag-C**, according to the Magnetofection and **EcoTransfect** instruction manuals. β-Galactosidase expression was revealed 48 hours after transfection using **OZ Biosciences' ONPG assay kit** (catalog # GO10001).*

Conclusions: The **CombiMag** reagent and its fluorescently labeled counterpart, **FluoMag-C** have identical transfection efficiency with either a polymer-based or lipid-based transfection reagents. Consequently, the labeling procedure did not affect the activity of the magnetic nanoparticles.

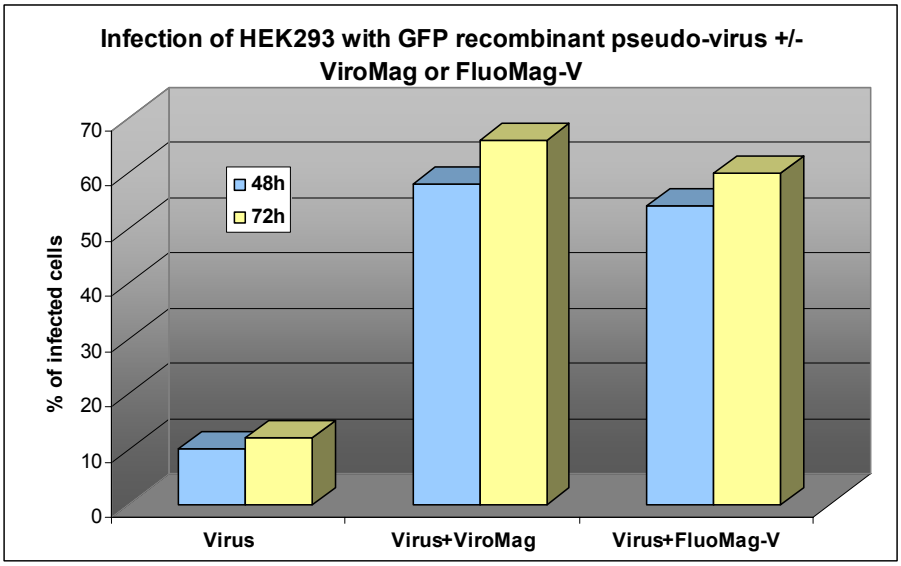
Gene Silencing Efficiency of FluoMag-S versus SilenceMag



*GFP stably transfected MDCK and HeLa cells were plated the day before transfection in a 24-well plate. Cells were then treated with **SilenceMag** or **FluoMag-S** and siRNA (targeting GFP or targeting LacZ as control) as described in the **SilenceMag** instruction manual. Complexes were prepared with 1 µl of **SilenceMag** or **FluoMag-S** and 10nM (67.5ng) of siRNA. Cells were then transfected in 500µl transfection volume. GFP expression level was monitored 72 h post-transfection by detection of fluorescence intensity with a fluorometer.*

Conclusions: The **SilenceMag** reagent and its fluorescently labeled counterpart, **FluoMag-S** have alike gene silencing efficiency. Consequently, the labeling procedure did not affect the activity of the magnetic nanoparticles for delivering siRNA.

Transduction Efficiency of FluoMag-V versus ViroMag



*HEK 293 cells were plated the day before infection in a 24-well plate. Cells were infected with 1 MOI either in presence of 2.5 µL of **ViroMag** or 2.5 µL **FluoMag-V**. After 15 min **Magnetofection** as indicated in **ViroMag** protocol, cells were placed in a 5% CO_2 incubator at 37°C. Culture medium was replaced 16h post-infection by fresh culture medium. GFP expression was analyzed by FACS 48h and 72h post-transduction.*

Conclusions: The **ViroMag** reagent and its fluorescently labeled counterpart, **FluoMag-V** have comparable transduction efficiency. Consequently, the labeling procedure did not affect the activity of the magnetic nanoparticles for assisting, controlling and promoting viral transduction.

Bibliographic References

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