

## NeuroMag Results

OZ Biosciences is pleased to present a new specific reagent for neurons transfection based on the Magnetofection™ technology: **NeuroMag**. It is specifically designed to achieve high transfection efficiency of primary neurons and neuronal cell lines. Magnetofection™ uses magnetic force to drive the nucleic acids associated with magnetic particles towards and into the target cells. In this way, the complete applied dose of nucleic acids gets concentrated onto the cells surface very rapidly so that 100% of the cells get in contact simultaneously with all DNA or RNA doses.

**NeuroMag** main features are:

1. High transfection efficiency
2. Specific for all primary neurons and neuronal cell lines
3. Concentrate the entire nucleic acids dose on the cells very rapidly
4. Suitable for primary neurons cultured from 7 up to 21 days (div)
5. Absolutely not toxic and serum compatible
6. Higher and longer transgene expression level (expression up to 7 days post-transfection)
7. Target/confine transduction to specific area (magnetic targeting)
8. Economical, Simple & Rapid
9. Amenable to high throughput automation

### Nucleic Acid Types

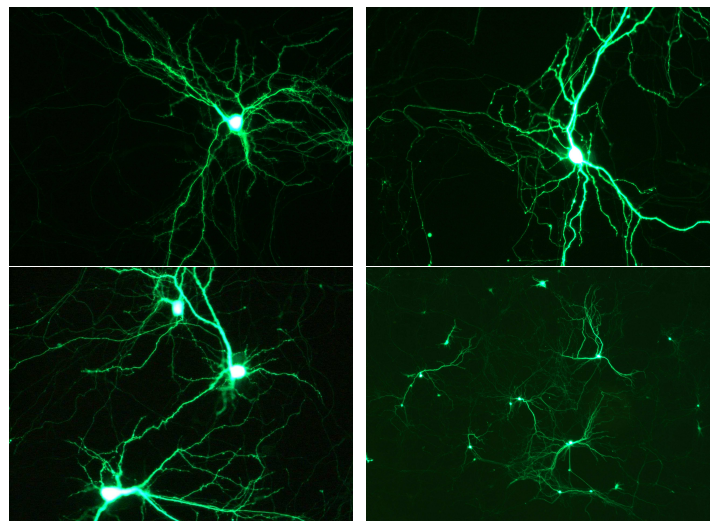
**NeuroMag** Transfection Reagent is suitable for all type of nucleic acids including: plasmid DNA, siRNA, oligonucleotides, linearized DNA, double stranded RNA, mRNA, shRNA.

### Cell Types

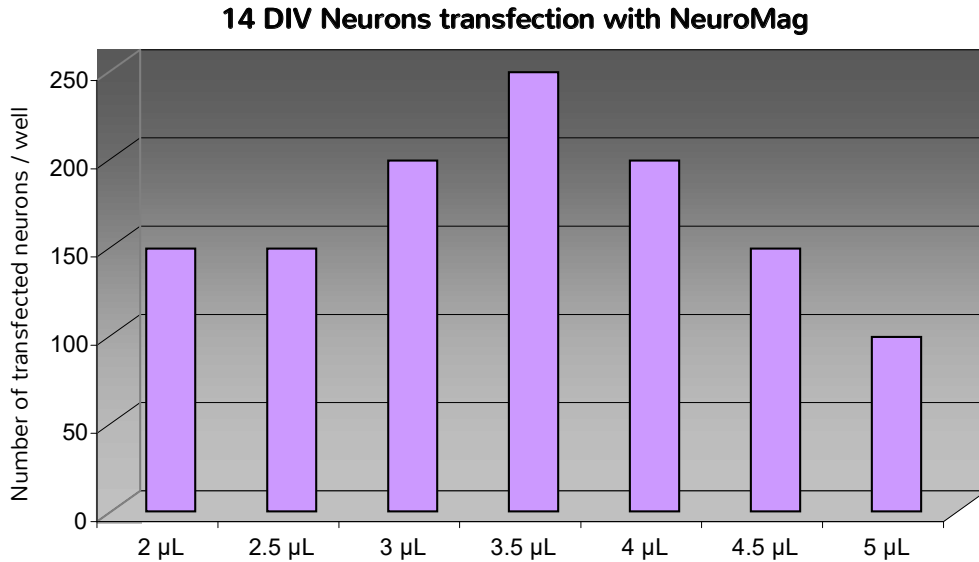
**NeuroMag** is applicable and has been tested successfully on primary hippocampal neurons and a variety of neuronal cell lines such as PC-12, B95, C6, N2A or SH-5YSY.

### Rat hippocampal primary neurons transfected with NeuroMag

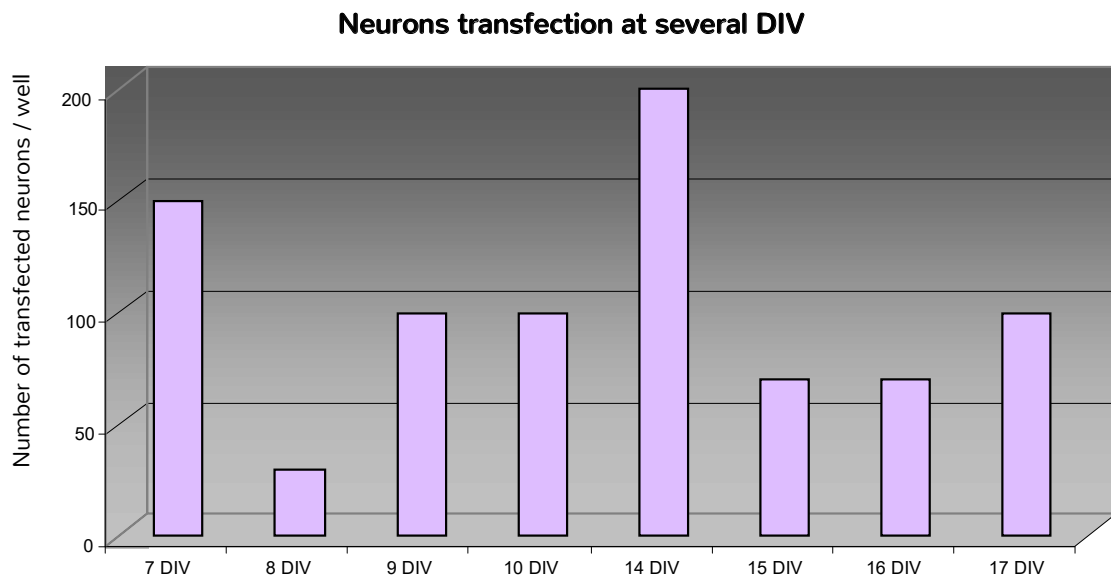
Primary rat hippocampal neurons were prepared in 24-well plates as described in the **NeuroMag** instruction manual. Cells were transfected after 14 DIV (Days *In Vitro*) using 1 µg / well of pEGFP plasmid and 3.5 µL of **NeuroMag**. Transfection efficiency was monitored by fluorescence microscopy 48 h post-transfection.



**Optimization of rat hippocampal primary neurons transfection**

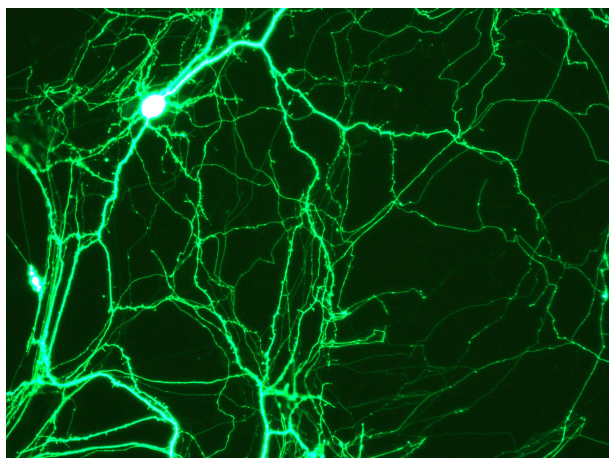


Rat hippocampal primary neurons were prepared in 24-well plates as described in the NeuroMag protocol. Cells were transfected after 14 DIV (Days *In Vitro*) using 1 µg / well of pEGFP plasmid and various amounts of **NeuroMag** reagent. Transfection efficiency was monitored by fluorescence microscopy 48 h post-transfection.

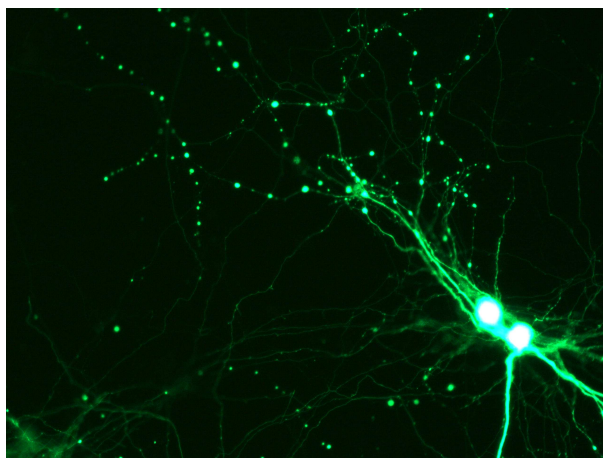


Rat hippocampal primary neurons were prepared in 24-well plates as described in the NeuroMag protocol. Cells were transfected at several DIV using 1 µg / well of pEGFP plasmid and 3.5 µL of **NeuroMag**. Transfection efficiency was monitored by fluorescence microscopy 48 h post-transfection.

## NeuroMag is not toxic for primary neurons



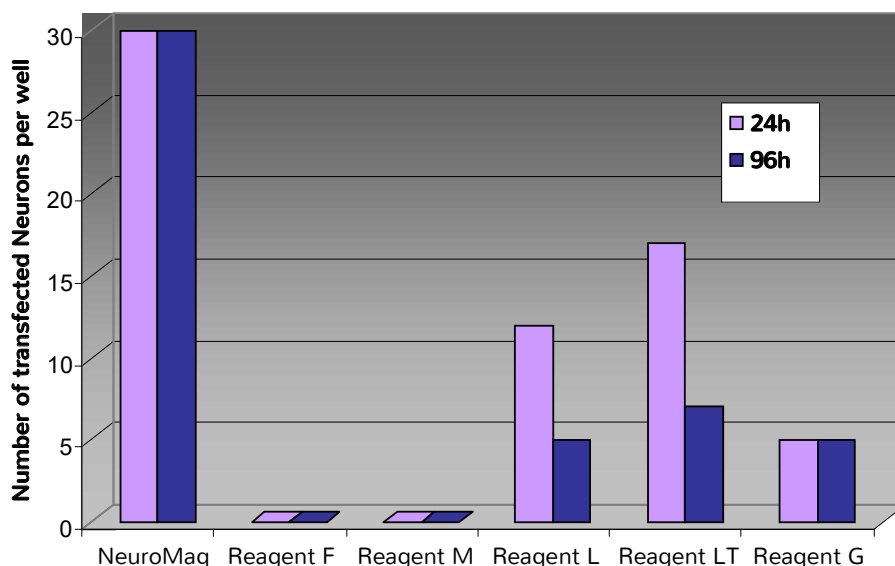
Primary neurons transfected with **NeuroMag**



Primary neurons transfected with **reagent LP**

Rat hippocampal primary neurons were prepared in 24-well plates as described in the **NeuroMag** protocol. Cells were transfected after 14 DIV using 1  $\mu\text{g}$  / well of pEGFP plasmid and 3.5  $\mu\text{L}$  of **NeuroMag** or using the reagent LP according to the manufacturer instructions. Transfection efficiency was monitored by fluorescence microscopy 72 h post-transfection.

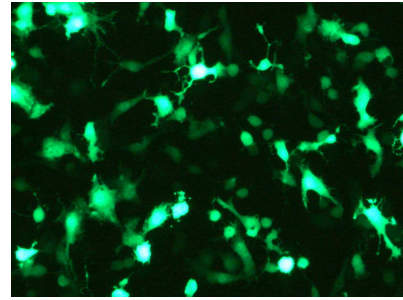
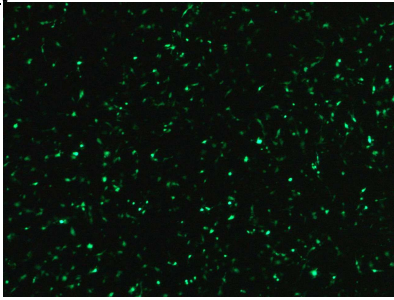
## Comparison with other commercially available transfection reagents



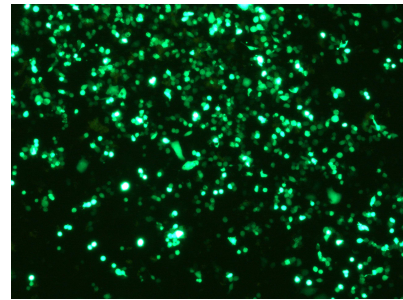
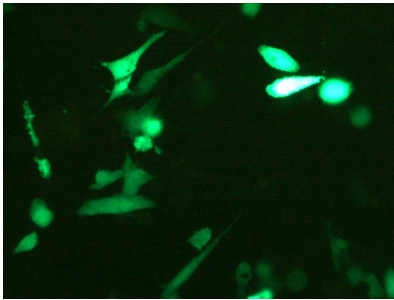
Rat hippocampal primary neurons were prepared in 24-well plates as described in the **NeuroMag** protocol. Cells were transfected after 15 DIV using 1  $\mu\text{g}$  / well of pEGFP plasmid and 3.5  $\mu\text{L}$  of **NeuroMag** or using competitor's transfection reagent according to the manufacturer's manuals. Transfection efficiency was monitored by fluorescence microscopy at 24 h or 96 h post-transfection.

*Other brain related cells transfected with NeuroMag*

1- **Efficiency:**

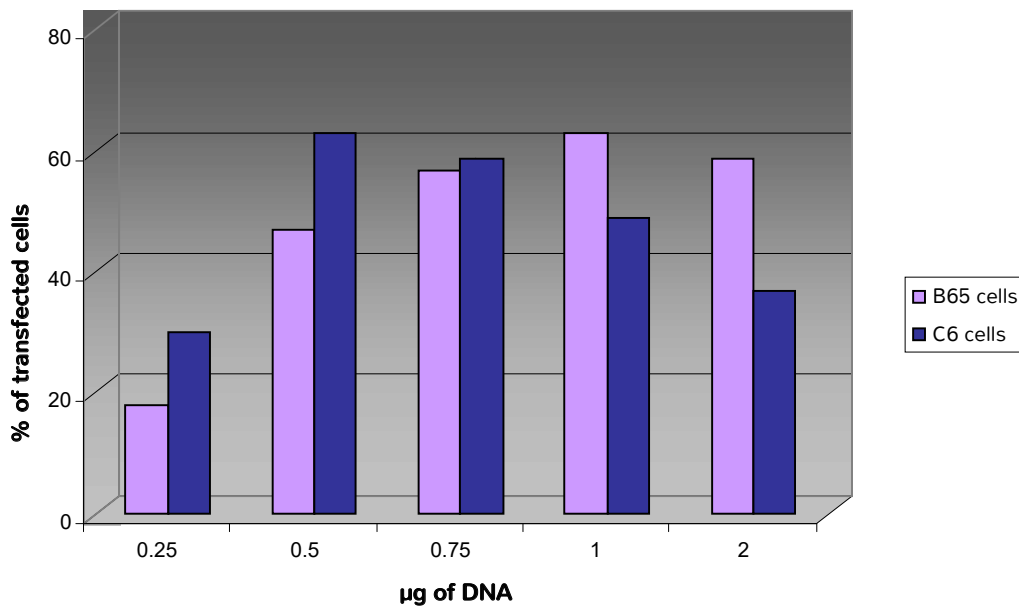


C6 glial cells transfected with 1 µg of pEGFP plasmid DNA

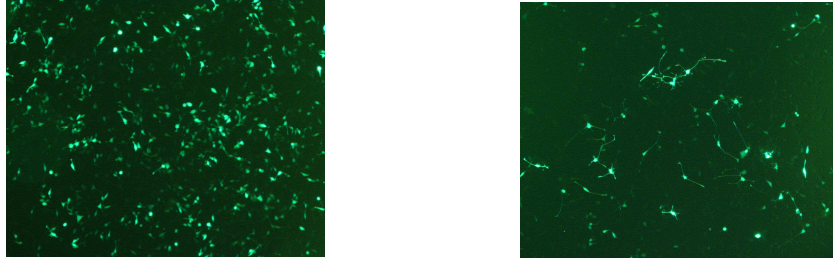


B65 cells transfected with 1 µg of pEGFP plasmid DNA

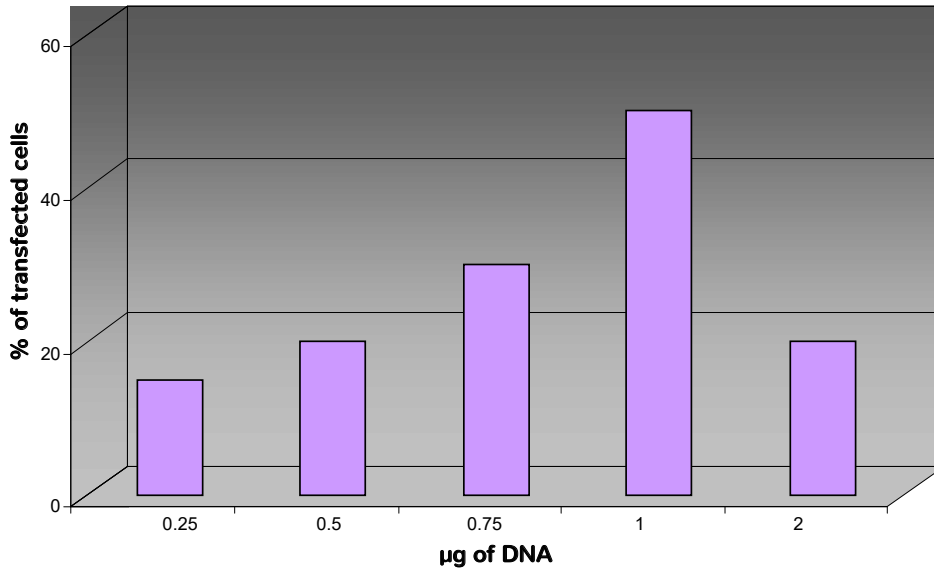
N2A transfected with 1 µg of pEGFP plasmid DNA



B65 neurons and C6 glial cells were transfected in 24-well plates with various amounts of pEGFP plasmid and 3.5 µL of **NeuroMag**. Transfection efficiency was monitored by FACS 24 h post-transfection.

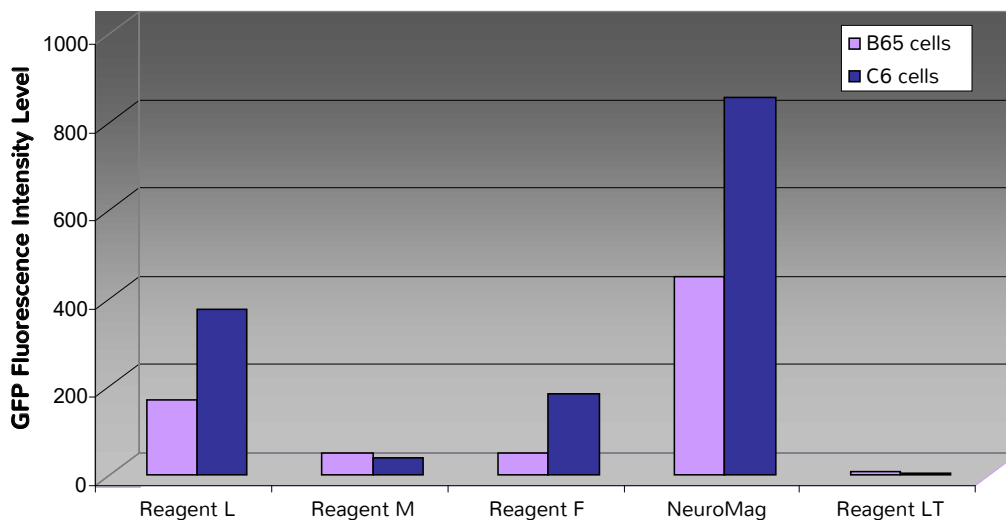


NGF (Nerve Growth Factor) differentiated PC12 cells transfected with 1 µg of pEGFP plasmid DNA

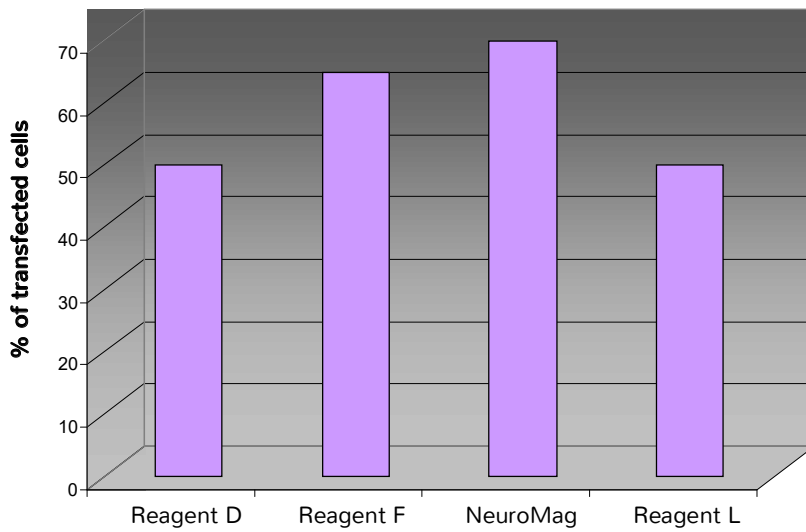


NGF (Nerve Growth Factor) differentiated PC12 cells were transfected in 24-well plates with various amounts of pEGFP plasmid and 3.5 µL of **NeuroMag**. Transfection efficiency was monitored by FACS 24 h post-transfection.

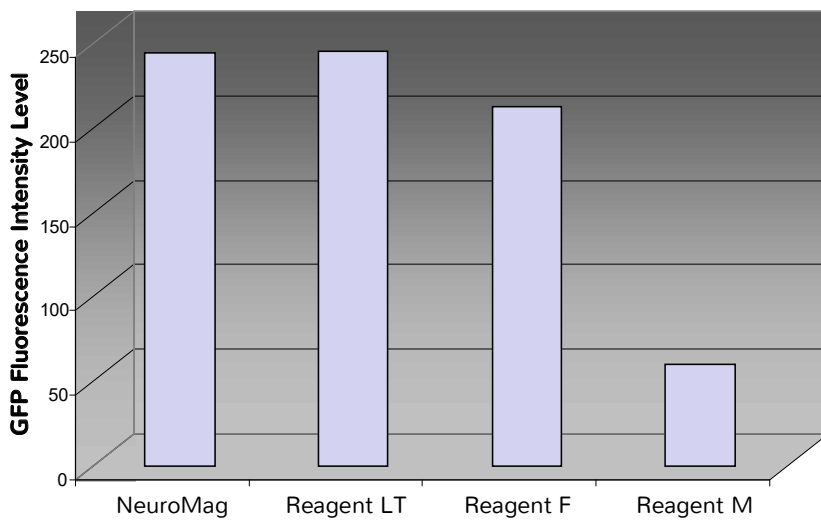
## 2- Comparison with competitor's transfection reagent:



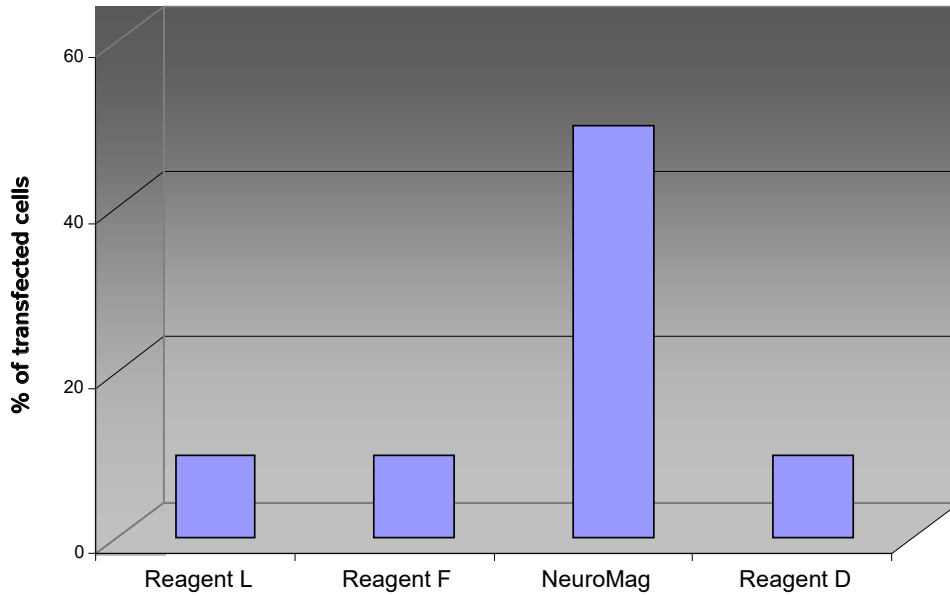
B65 neurons and C6 glial cells were transfected with 1 µg / well of pEGFP plasmid and 3.5 µL of NeuroMag or with competitor's transfection reagent according to the manufacturer's manuals. Transfection efficiency was monitored by fluorescence microscopy and FACS 24 h and 96 h post-transfection.



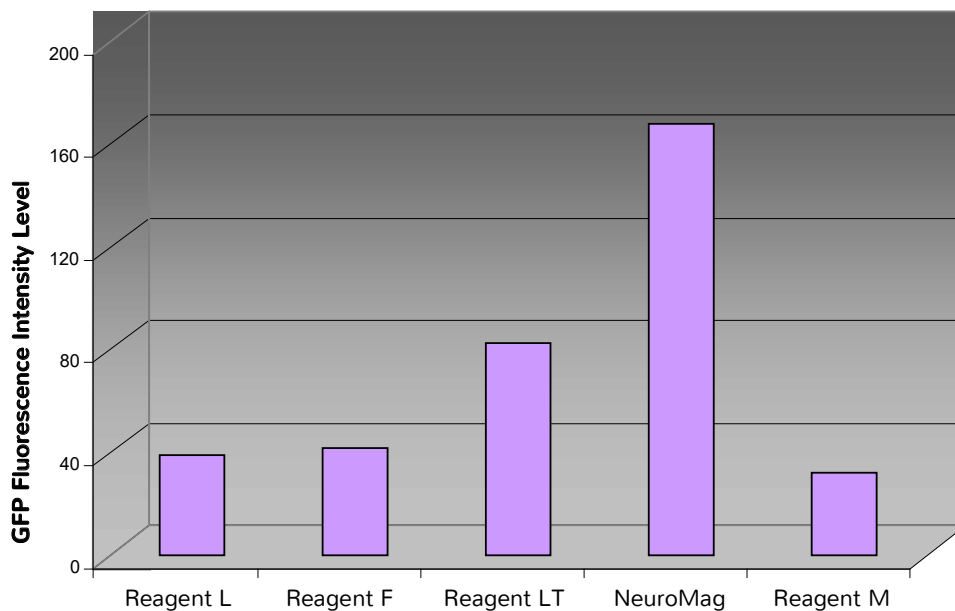
N2A cells were transfected in 24-well plates with 1  $\mu$ g of pEGFP plasmid and 3.5  $\mu$ L of NeuroMag or with competitor's reagent as recommended in their instruction manual. Transfection efficiency was monitored by FACS 24 h post-transfection.



SH-5YSY cells were transfected in 24-well plates with 1  $\mu$ g of pEGFP plasmid and 3.5  $\mu$ L of NeuroMag or with competitor's reagent as recommended in their instruction manual. Transfection efficiency was monitored by FACS 24 h post-transfection.



NGF (Nerve Growth Factor) differentiated PC12 cells were transfected with 1  $\mu\text{g}$  / well of pEGFP plasmid and 3.5  $\mu\text{L}$  of NeuroMag or with competitor's transfection reagent according to the manufacturer's manuals. Transfection efficiency was monitored by fluorescence microscopy and FACS 24 h and 96 h post-transfection.



NGF (Nerve Growth Factor) differentiated PC12 cells were transfected with 1  $\mu\text{g}$  / well of pEGFP plasmid and 3.5  $\mu\text{L}$  of NeuroMag or with competitor's transfection reagent according to the manufacturer's manuals. Transgene protein expression level was monitored with a CytoFluor multiplate reader 96 h post-transfection. This clearly demonstrates the absence of NeuroMag cytotoxicity related to high transgene expression level.