

ViroMag Stem Results.

1. Description

ViroMag Stem, a Magnetofection-based Lentiviral Transduction Enhancer, enables improved viral driven genetic modification in a wide range of stem cells in preclinical and clinical applications, such as ex vivo gene therapy and cell therapy.

ViroMag Stem, as a stabilized magnetic nanoparticles formulation, offers a simple and reproducible method for increasing **lentiviral infection** and **transduction of difficult cell types** such as CD34+ hematopoietic stem cells, both cell lines and primary cells, in cell culture plate using the Magnetofection™ technology.

This method combines maximum cell viability and high transduction efficiency while cell phenotype and differentiation potential are not affected.

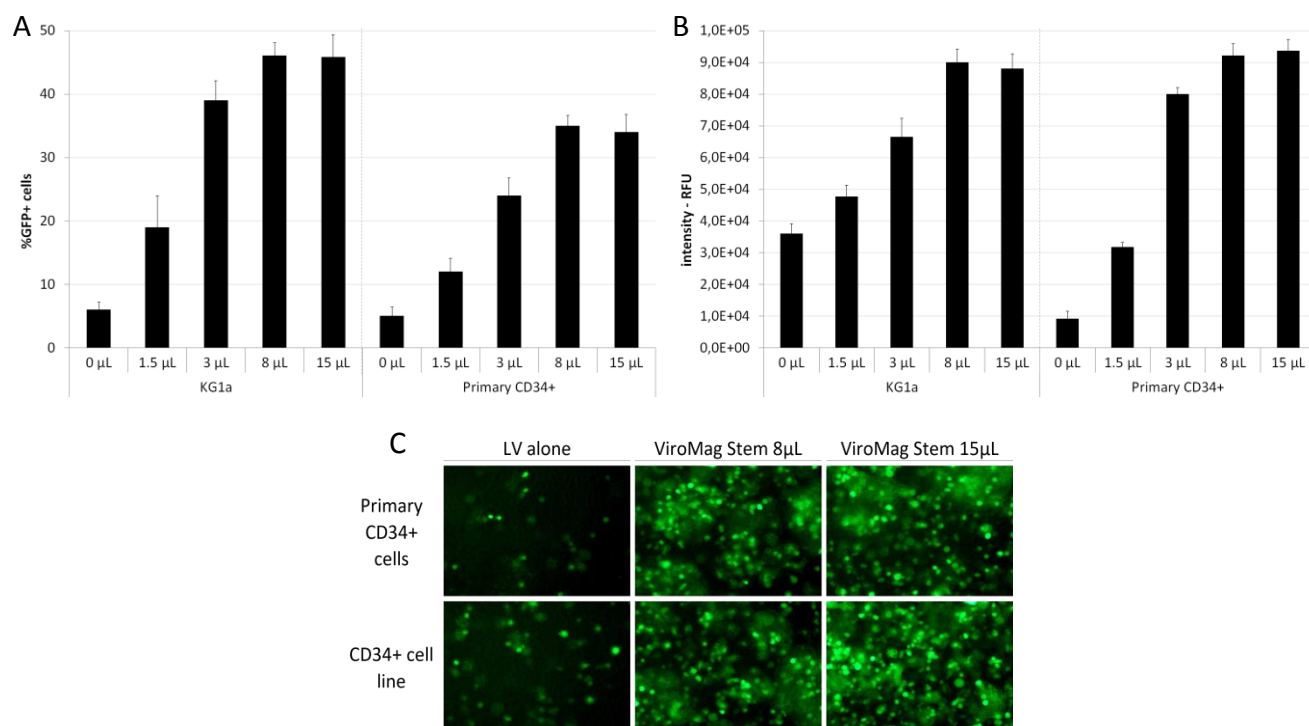
2. Storage and shipping condition

Storage & Shipping: Upon reception and for long-term use, store Tube ST at -20°C and Tube VM at +4°C - Stability: 1 year. The reagent is shipped at RT.

ViroMag Stem enhances Lentiviral infection in CD34+ stem cells.

ViroMag Stem increases infection and transduction efficiency in primary CD34+ cells and CD34+ cell lines.

KG1a CD34+ cell lines and CD34+ primary cells were infected at MOI 5 (5 viral particles per cell) using a HIV-SFFV-GFP lentivirus in presence of ranging doses of ViroMag Stem WS. 72 hours after transduction, % of GFP positive cells (A) and total intensity (B) were analyzed by flow cytometry and fluorescence microscopy (C).

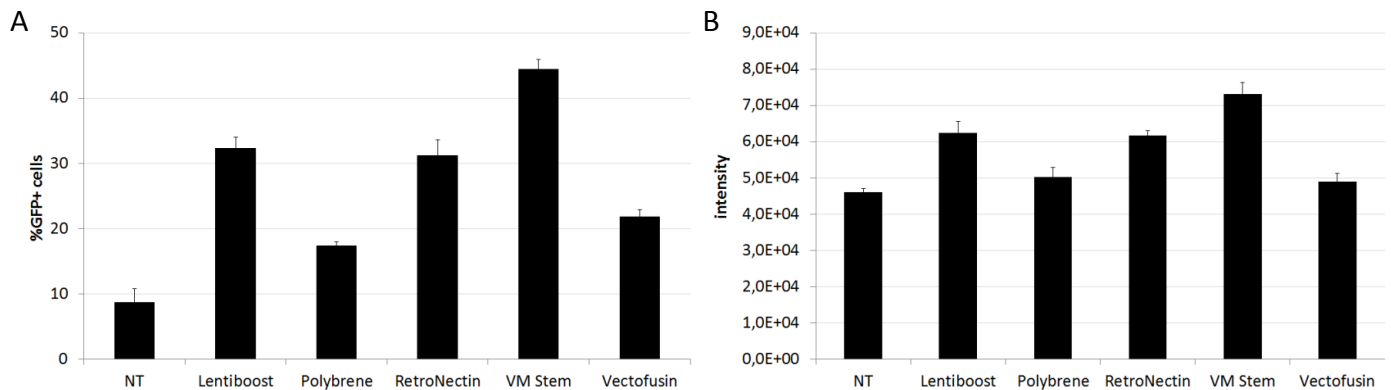


These results demonstrated the capacity of ViroMag Stem to enhance infection and transduction in CD34+ stem cells.

ViroMag Stem compared to competitors for Lentiviral infection in CD34+ stem cells.

ViroMag Stem outperforms lentiviral mediated transduction efficiency in CD34+ cell lines.

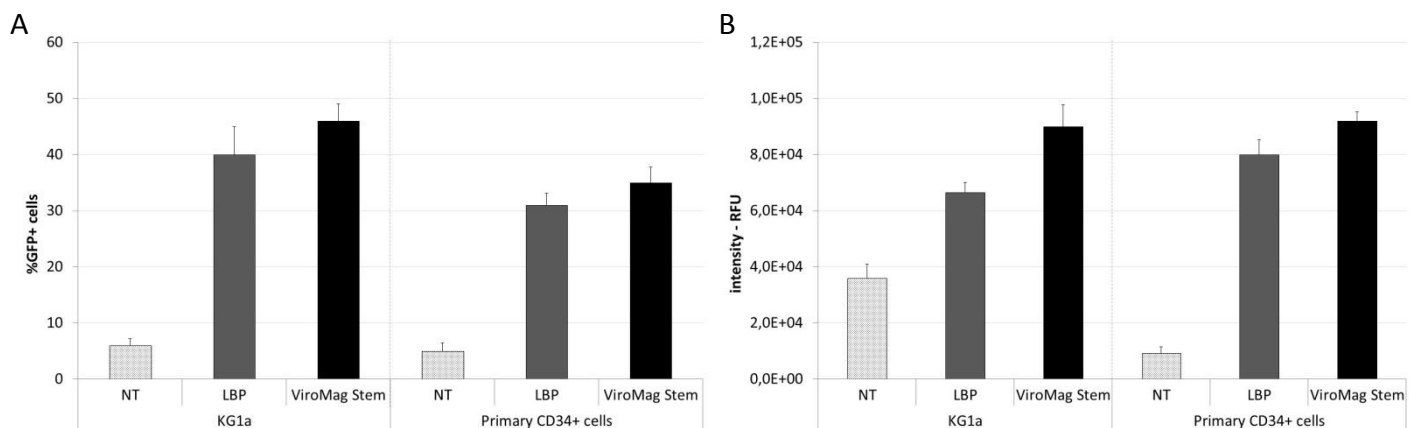
KG1a CD34+ cells were infected at MOI 5 (5 viral particles per cell) using a HIV-SFFV-GFP lentivirus in presence of viral enhancers at the recommend doses. 72 hours after transduction, % of GFP positive cells (A) and total intensity (B) were analyzed by flow cytometry.

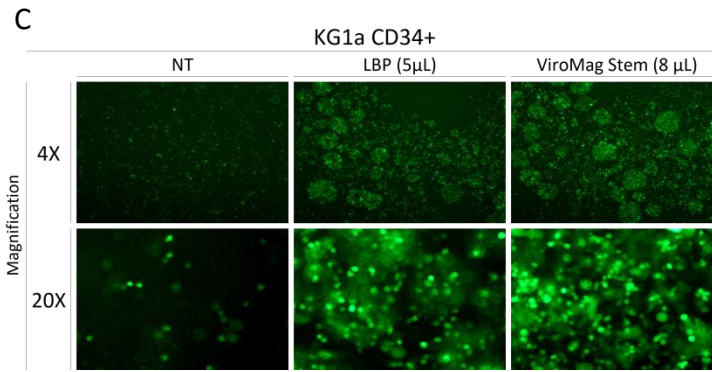


ViroMag Stem vs LentiBlast Premium for CD34+ transduction.

ViroMag Stem enhances infection and transduction of CD34+ cells compared to LentiBlast Premium.

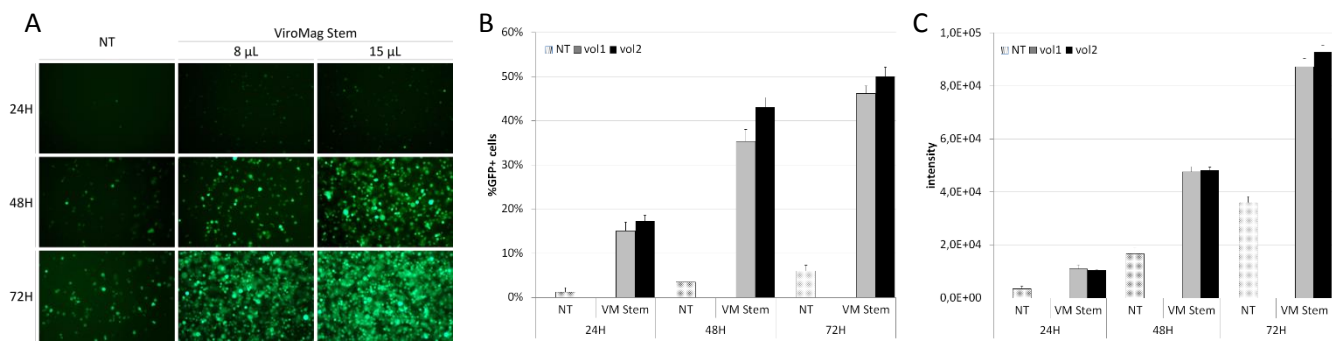
KG1a CD34+ cell lines and CD34+ primary cells were infected at MOI 5 (5 viral particles per cell) using a HIV-SFFV-GFP lentivirus in presence of 5 μ L of LentiBlast Premium (LBP) or 8 μ L of ViroMag Stem WS. 72 hours after transduction, % of GFP positive cells (A) and total intensity (B) were analyzed by flow cytometry and fluorescence microscopy (C).





ViroMag Stem improves infection and transduction of CD34+ cells compared to LentiBlast Premium over 3 days.

KG1a CD34+ cell lines were infected at MOI 5 using a HIV-SFFV-GFP lentivirus in presence of 5 µL or 10 µL of LentiBlast Premium (LBP) and 8 µL or 15 µL of ViroMag Stem WS (VM Stem), respectively vol 1 and vol 2 for each transduction enhancer. Fluorescence expression was monitored after 24H, 48H and 72 hours by fluorescence microscopy (A) or flow cytometry for % of transfected cells (B) or intensity (C).

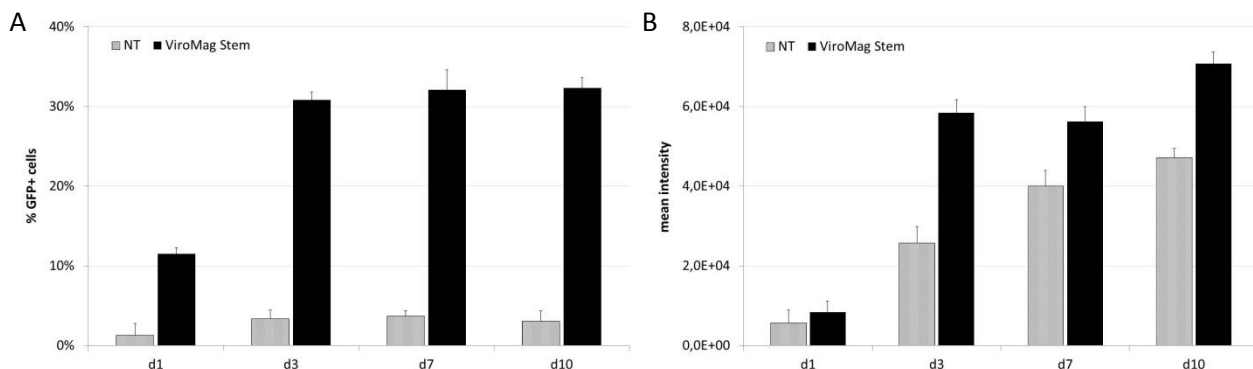


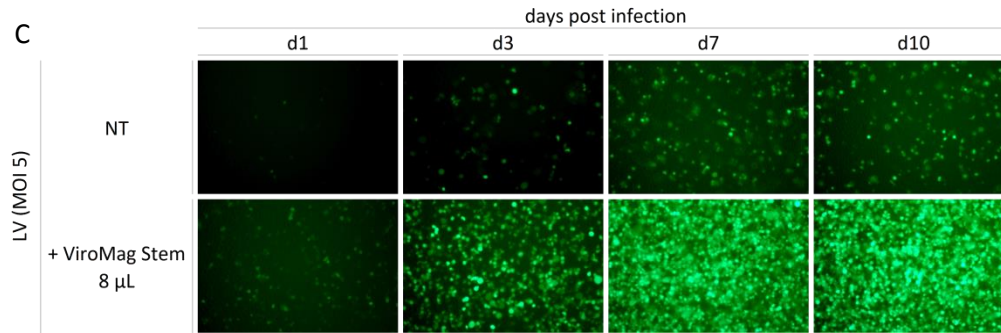
Results demonstrated that ViroMag Stem succeeded in increasing infection and transduction in CD34+ cell line compared to LBP.

ViroMag Stem: kinetics of transduction over 10 days.

ViroMag Stem improves lentivirus-induced genetic modification of CD34+ cells over 10 days.

CD34+ stem cell line KG1a was transduced with Lentivirus encoding for GFP at MOI of 5 in presence or not of 8 µL ViroMag Stem WS. % of GFP positive cells (A) and mean intensity (B) of genetically modified cells were evaluated by flow cytometry and transduction efficiency was visualized under fluorescence microscopy (C) over 10 days (d1 to d10).



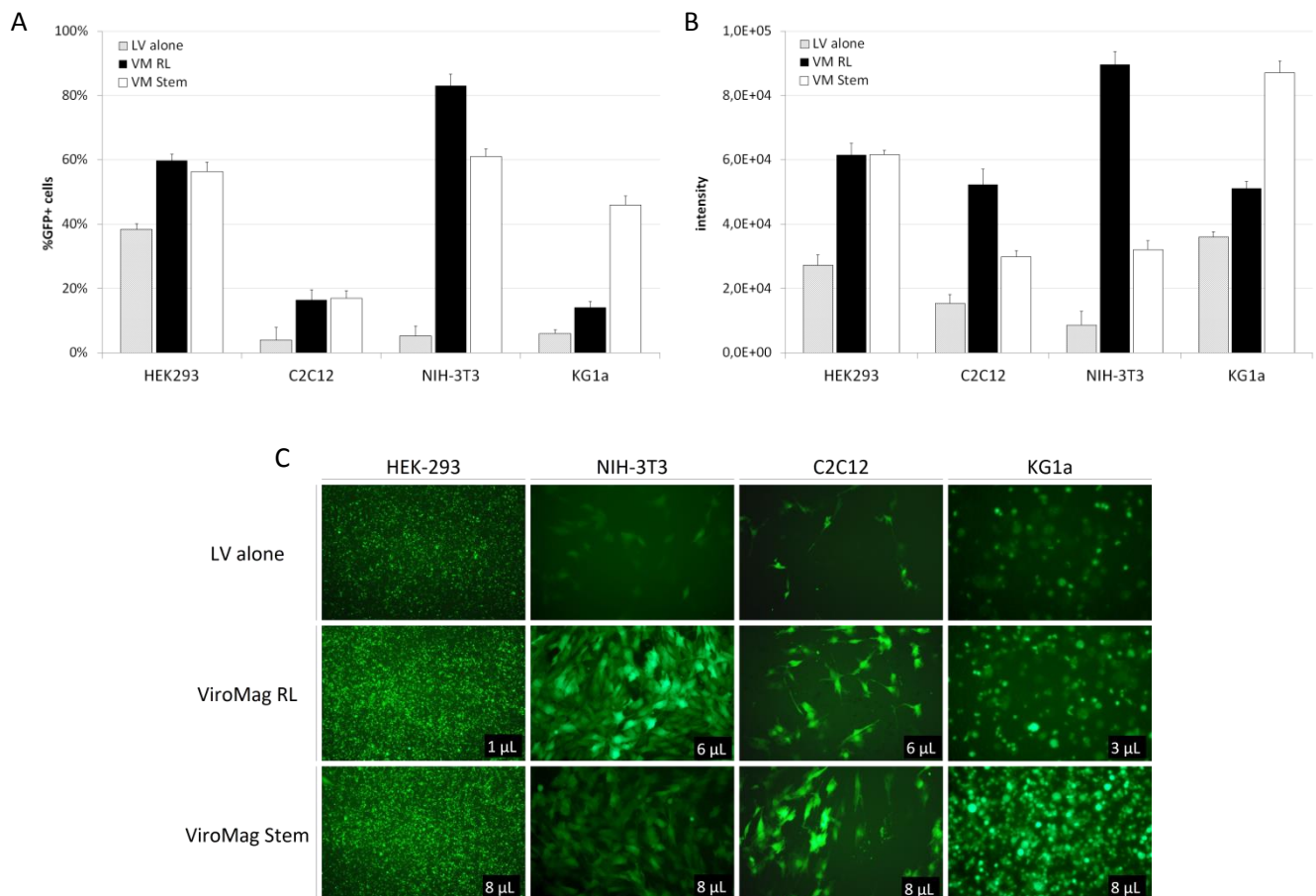


Results demonstrated that ViroMag Stem dramatically increases the transduction efficiency of lentiviral particles in KG1a over 10 days of infection.

ViroMag Stem: Specificity for CD34+ Stem cells

ViroMag Stem more specific for CD34+ cells than for other cell lines.

HEK293T, C2C12, NIH-3T3 and KG1a cell lines were transduced Lentivirus encoding for GFP in presence or not of 8 μL of ViroMag Stem WS or various doses of ViroMag RL. After 72 h incubation, % of GFP positive cells (A) and mean intensity (B) of genetically modified cells were evaluated by flow cytometry and transduction efficiency was monitored under fluorescence microscopy (C).

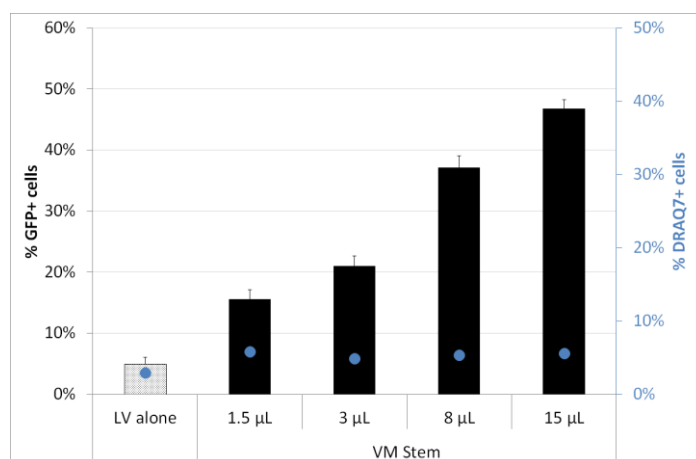


Results demonstrated that depending on cell lines, ViroMag Stem did not enhance infection or transduction as high as ViroMag RL did ; neither % nor protein production were improved with ViroMag Stem compared to ViroMag RL rendering ViroMag Stem specific for Stem cells.

CD34+ cells viability following ViroMag Stem-induced transduction

ViroMag Stem does not impair CD34+ stem cells survival after lentiviral mediated transduction.

KG1a CD34+ cell lines were infected at MOI 5 using a HIV-SFFV-GFP lentivirus in presence of ranging doses of ViroMag Stem WS (VM Stem). 72H after transduction, late apoptosis was measured using non-permeant DRAQ7 nuclear stain; % of GFP+ (bars) and DRAQ7+ (blue squares) cells were determined by flow cytometry.



Results demonstrated that ViroMag Stem does not induce toxicity after transduction.