Viro-MICST™ Results

i-MICST™ Technology (**I**ntegrated **M**agnetic **I**mmuno-cell **S**orting and **T**ransfection/**T**ransduction) is a new platform that allows to genetically modifying cells directly on magnetic cell purification columns or with magnetic cell sorting systems. This technology combines cell isolation and genetic modification in one simple, efficient and reliable integrated system.

Viro-MICST™ reagent is a new specific nanoparticles formulation issued from our Magnetofection™ Technology allowing high transduction efficiency with low Multiplicity of Infection (MOI) during magnetic cell separation. It is specially designed to be combined with all type of viruses. **Viro-MICST™** is a unique reagent offering a solution for such applications.

i-MICST™ Technology requires magnetic cell separation systems (not provided by OZ Biosciences) and **Viro-MICST™** nanoparticles reagent for capturing virus and infecting cells within the cell purification column.

Magnetic cell purification and transduction in one integrated system*

Main features are:

1. Isolation and transduction of cells in one reliable integrated system
2. High transduction efficiency
3. Rapid, simple and ready-to-use
4. Cell phenotype maintained
5. Synchronize viral adsorption and accelerate the transduction process
6. Infect non permissive cells
7. Suitable for all viruses and all cells

***** *For detailed information, please refer to Viro-MICST™ protocol.*

Cell types

Viro-MICST™ has been successfully tested on a variety of immortalized cell lines and primary cells. Please consult our updated list of cells successfully tested available on the website: www.ozbiosciences.com
This reagent is generally applicable to all cells, but if a particular cell type is not listed, this does not imply that **Viro-MICST™** is not going to work. OZ Biosciences is going to frequently update this list. You can also submit your data to tech@ozbiosciences.com so we can revise this list.

Table 1: Cells successfully transduced with **Viro-MICST™**.

Primary Cells	Cell Type	Source
hUC-MSCs	Umbilical cord mesenchymal stem cells	Human
hCB-HSCs	Cord blood hematopoietic stem cells	Human
Sca-1 + /LSK+	Sca-1 + haematopoietic stem cells	Mouse
hPBMC	Peripheral blood mononuclear cells	Human
CD4+, CD8+	T lymphocytes	Macaque
Cell Lines	**Cell Type**	**Source**
293, HEK-293, 293-T, -EBNA	Embryonic kidney epithelial cells	Human
CHO	Chinese Hamster Ovary cells	Hamster
HMEC-1	Microvascular endothelial cell line	Human
Jurkat, CEMx174, H9	T lymphocytes	Human
K562	Erythromyeloblastoid leukemia cell line	Human
KOPN	Ph1-positive leukemia cell lines	Human
RAW 264.7	Macrophages	Mouse
U937	Leukemic monocyte lymphoma	Human

Virus types

Viro-MICST™ is suitable for all type of viruses including: AAV, adenovirus, lentivirus and retrovirus. If a particular virus is not listed, this does not imply that **Viro-MICST™** is not going to work. OZ Biosciences is maintaining an updated list of virus successfully tested that is available on the website: www.ozbiosciences.com.

Table 2: Virus successfully tested with **Viro-MICST™**.

Virus type	Virus name
Adenovirus/ Adeno-Associated Virus	Ad5-LacZ/-GFP, Ad5-PEG
Lentivirus / Retrovirus	HIV, SIV ,MuLV, MLV, FIV
Herpes virus	HSV-I
Alpha virus	Sindbis virus
Rhabdovirus	VSV
Polyomavirus	SV40
Paramyxovirus	Measles

Cell separation technologies

OZ Biosciences has developed Viro-MICST™ in association with MACS® technology* from Miltenyi Biotec (www.miltenyibiotec.com). Results and demonstration were performed on MS and LS columns with MACS® separators and cell separation reagents according to MACS® protocol. Accordingly, the Viro-MICST™ protocol is adapted to MACS® columns. i-MICST™ is also apt for other magnetic cell separation technologies.

**MACS® is a registered trademark owned by Miltenyi Biotec GmbH and the use of MACS® column is proprietary and patented technology. For any further licensed of MACS® system, please contact Miltenyi.*

i-MICST™ technology principle

i-MICST™ Technology requires magnetic cell separation systems (not provided by OZ Biosciences) and **Viro-MICST™** reagent for capturing virus and infecting cells within the magnetic cell purification column. **Viro-MICST™** was developed in association with MACS® technology* from Miltenyi Biotec.

Viro-MICST™ does not interfere with magnetic cells sorting and we recommend following rigorously the cell sorting protocol given by the manufacturer. In most cases, magnetic cells sorting procedure requires the use of at least two purification columns to achieve high purification efficiency. We recommend using two purification columns and to apply the **Viro-MICST™** reagent complexed to the viruses (transduction procedure) on the second column or the last column if more than 2 columns are required (see procedure below).

Critical parameters for cell enrichment are:

a) The percentage of the target cell population to be purified
b) The degree of the target cell population purity expected

We suggest using two magnetic columns if:
- The cell population to be purified represents less than 50 % of the total cell population
- The degree of purity needs to be above 90 %.

The first non-modified column will be used for pre-enrichment of the target cell population and the second column will be modified with complexes to increase cell purification while transducing the cells.

The **Viro-MICST™** protocol is depicted as a two-steps process.

– The first step (Figure 1) consists in a pre-enrichment of the target cell population that is only required if the percentage of the cell population to be purified and infected represents less than 50% of the total cell population and/or if the degree of purity to be reached is above 90%. OZ Biosciences does not provide magnetic cell separation systems, please refer to the manufacturer instructions protocols for this step.

– The second step (Figure 2) mainly consists in reaching high purity and simultaneously infecting the target cell population.

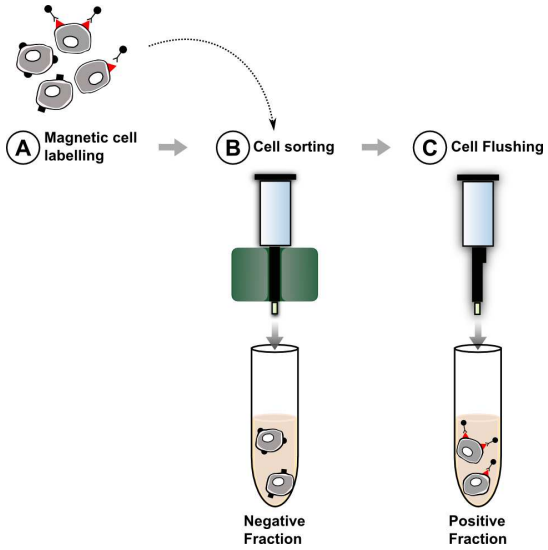


Figure 1. Pre-enrichment of the target cell population Using specific antibody-magnetic beads (A), cells are sorted on the magnetic column according to MACS® protocol (B). Only cells presenting target antigen are maintained on the magnetic device. After washing, a flushing procedure (C) allows to recover the target cell population. **MACS® is a registered trademark owned by Miltenyi Biotec GmbH and the use of MACS® column is proprietary and patented technology. For any further licensed of MACS® system, please contact Miltenyi.*

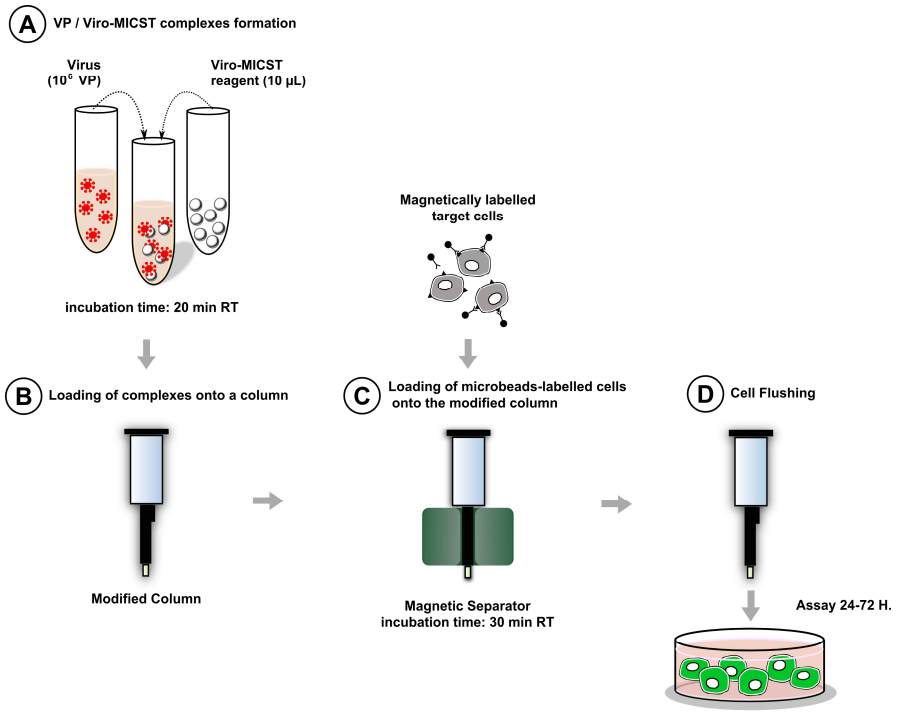


Figure 2. Schematic representation of the Viro-MICST™ procedure. After 20 min incubation, complexes of virus and **Viro-MICST™** reagent (A) are loaded onto the column according to the void volume of the column (B). Cell sorting is then performed as described by the manufacturer protocol. Cells are maintained in the column under magnetic field (C). At this step, transduction occurs. After washing, a flushing procedure allows to recover transfected sorted cells (D).

Viro-MICST™: high level of target cell transduction during purification

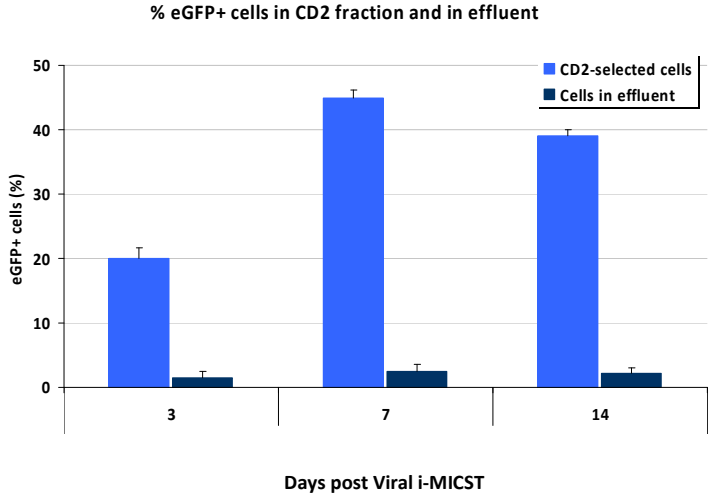


Figure 3: A mixture of $0.5x10^6$ Jurkat T cells and $0.5x10^6$ K562 cells was treated with CD2 microbeads and loaded into one MACS® LS column followed by a second LS column modified with Viro-MICST/LV.eGFP complexes (MOI 0.5, 6.5 µL Viro-MICST). The CD2- cell fraction in the effluent (K562 cells) and the CD2+ cells positively selected within the column (Jurkat cells) were analyzed for GFP expression at different time point [*].

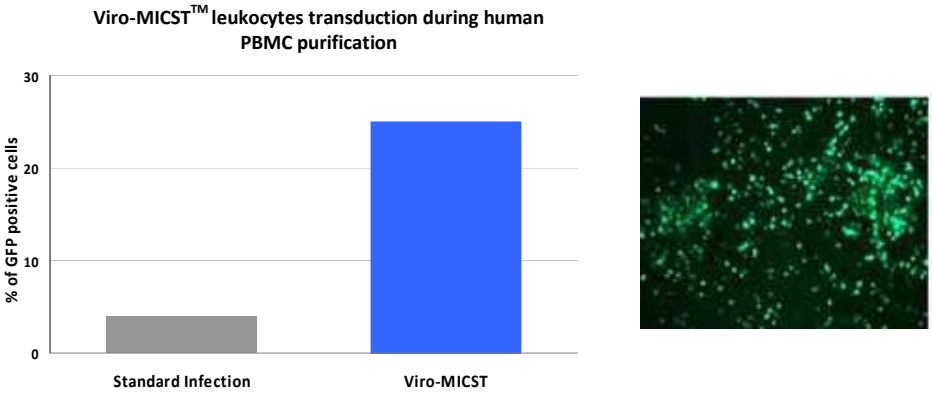


Figure 4: Human PBMC (extracted from whole blood) were labeled with CD45 microbeads then loaded onto:

i) one unmodified MACS® LS column and then transduced with lentivirus under standard protocol with a MOI of 0.5 (standard infection) or

ii) one unmodified LS column followed by one modified LS column with Viro-MICST/ LV.eGFP complexes with a MOI of 0.5 and 5 µL of Viro-MICST reagent per 10^6 infectious particles.

Transduction efficiency was measured by flow cytometry. Picture represents CD45+ (Leukocytes) PBMCs infected with the Viro-MICST procedure (10x magnification) [*].

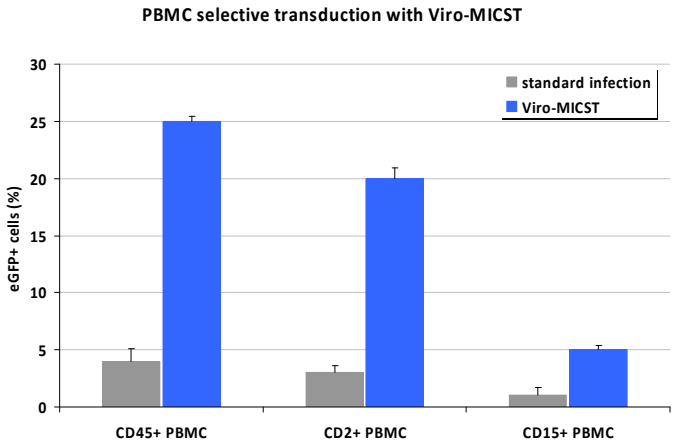


Figure 5: Human PBMC (extracted from whole blood) were labeled with either CD45, CD2 or CD15 microbeads then each condition were loaded into:
i) one unmodified LS column, and selected cells were then infected using standard lentiviral protocol with a MOI of 0.5 (standard infection) or
ii) one unmodified LS column followed by a LS column modified with Viro-MICST/ LV.eGFP complexes formulated at a MOI of 0.5 with 6.5 µL of Viro-MICST per 10^6 VP.-Infection efficiency was measured by flow cytometry [*].

These data show that Viro-MICST™ increases the transduction efficiency on non-adherent blood cells during purification step onto a modified cell sorting column.

Viro-MICST™ increases lentiviral transduction efficiency of stem cells

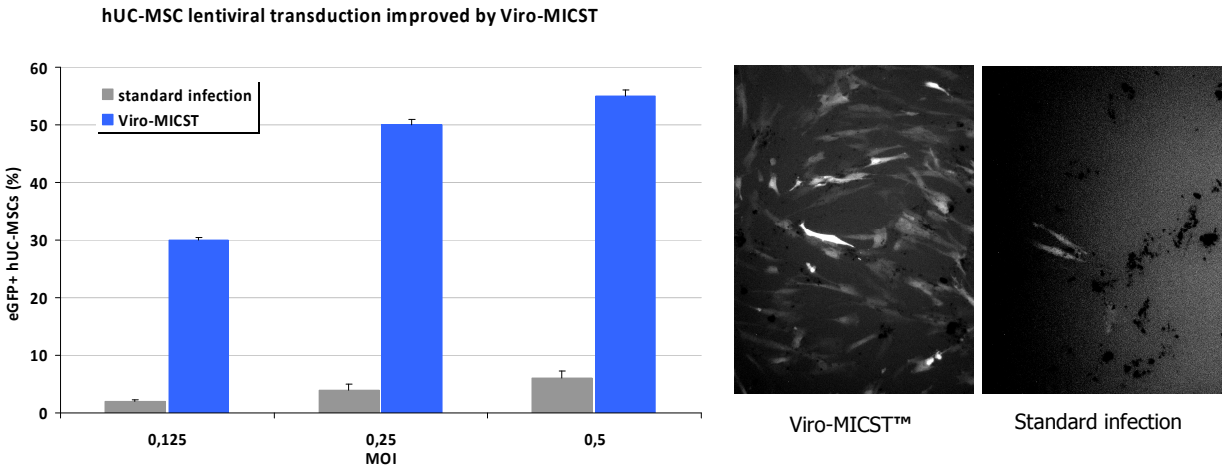


Figure 6: 1X10^6 hUC-MSC cells were transduced at different MOI using either standard lentiviral transduction procedure or Viro-MICST. For Viro-MICST, 1x10^6 hUC-MSC cells were labeled with CD105 microbeads and loaded into one Viro-MICST/ LV.eGFP modified LS column (13 µL per 10^6 VP). Transduction efficiency was measured by flow cytometry 3 days post-transduction. Images show hUC-MSCs 3 days after transduction with both procedures at a MOI of 0.5 (x10) [*].

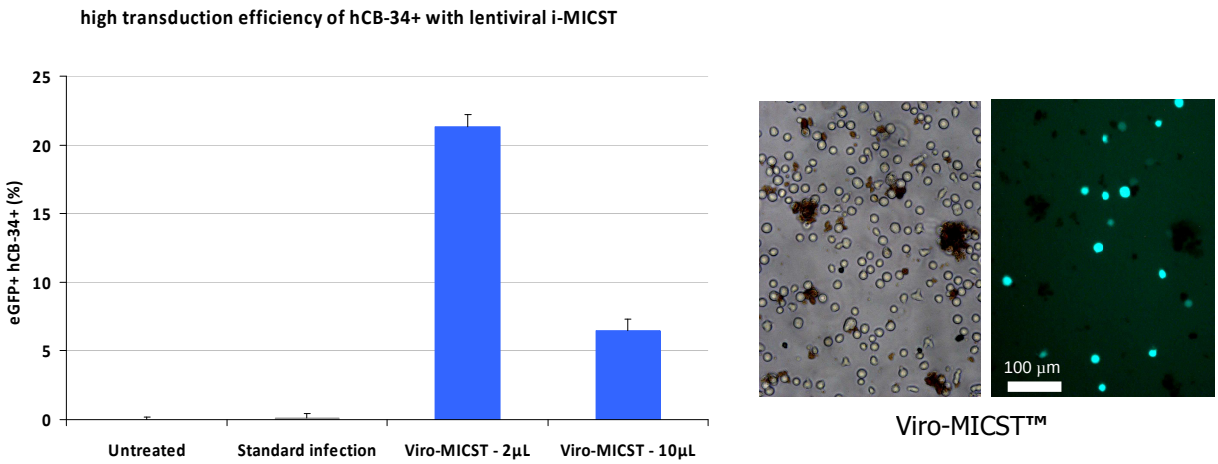


Figure 7: hCB-HSCs were transduced at a low cell density of 1.5 x 10^5 cells/mL without cell stimulation and at a MOI of 10 using either standard lentiviral infection procedure or Viro-MICST. For Viro-MICST, hCB-HSC cells were labeled with CD105 microbeads and loaded into one LS column modified with 2 or 10 µL Viro-MICST/ 10^6 LV.eGFP particles. Transduction efficiency was measured by flow cytometry 3 days post-infection. Photos show hCB-HSCs 2 days post-infection with a MOI of 4 (10X) [*].

Viro-MICST™ increases adenoviral transduction efficiency of stem cells

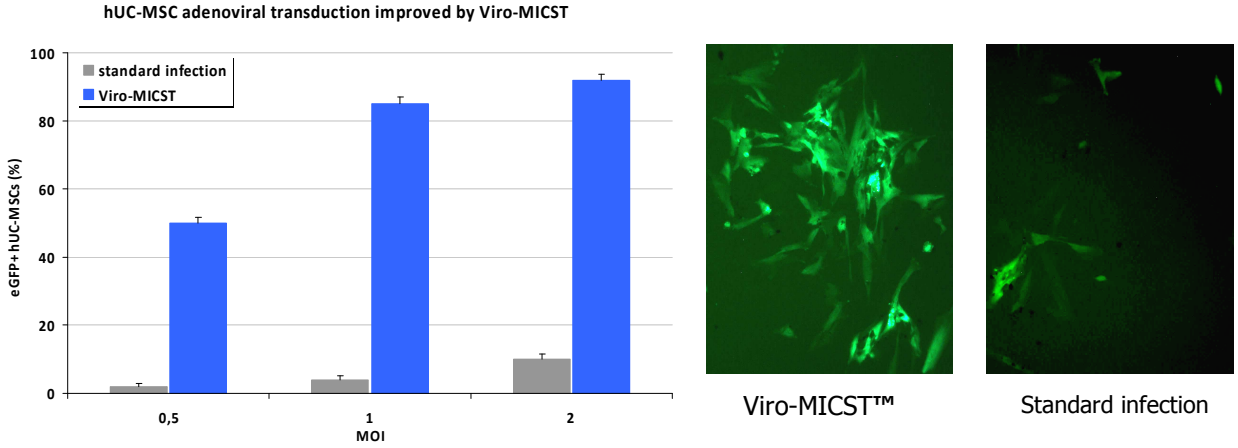


Figure 8: 1x10^6 hUC-MSC cells were transduced at different MOI using either standard adenoviral infection procedure or i-MICST: 1X10^6 hUC-MSC cells were labeled with CD105 microbeads and loaded into one LS column modified with Viro-MICST/ Ad.eGFP (3µL of Viro-MICST per 10^6 VP). Transduction efficiency was measured by flow cytometry 3 days post-infection. Pictures show hUC-MSCs 2 days after transduction with both procedures at a MOI of 2 (10X) [*].

Taking together the results demonstrated that Viro-MICST™ considerably enhanced stem cells transduction as compared to classic lentiviral or adenoviral transduction.

Virus/Viro-MICST complexes preloaded on column do not modify cell purification

The results presented below, indicate that the modification of magnetic cells sorting column with Viro-MICST/viruses complexes does not interfere with the purification process. MACS® versus Viro-MICST: Jurkat T cells purification.

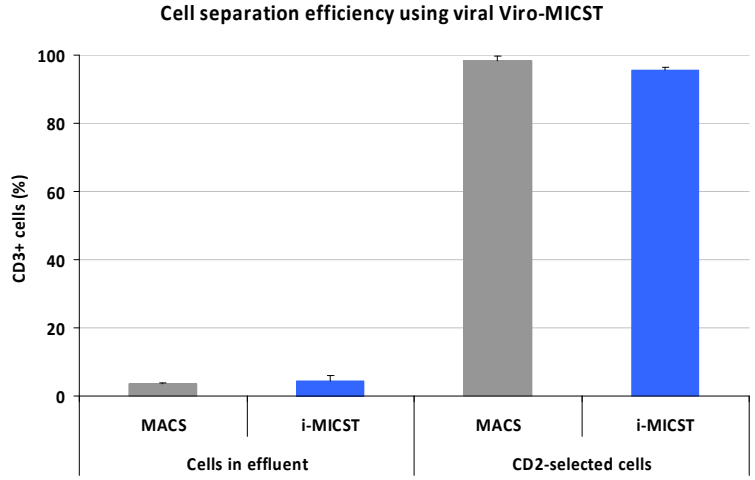


Figure 9: A mixture of 0.5x10^6 Jurkat T cells and 0.5x10^6 K562 cells were treated with CD2 microbeads and either passed *i*) sequentially through two MACS® LS columns (MACS procedure) or *ii*) through one MACS® LS column followed by i-MICST in the second LS column modified with Viro-MICST/lentivirus complexes (MOI:0.5; 5 µL Viro-MICST). Effect on cell purification was observed by analyzing the % of transduction in CD3+ cells retained on the column and the % of transduction in CD3- cells in the eluted fraction by Flow Cytometry [*****].

The same assay was conducted by loading 2.5x10^6 Jurkat T cells and 2.5x10^6 K562 cells in LS column. The results obtained were identical; no significant differences were observed between the regular MACS procedure and Viro-MICST procedure as described above (data not shown). The quantity of cells immuno-cell sorted and the purity of the cell population were the same with the two protocols.

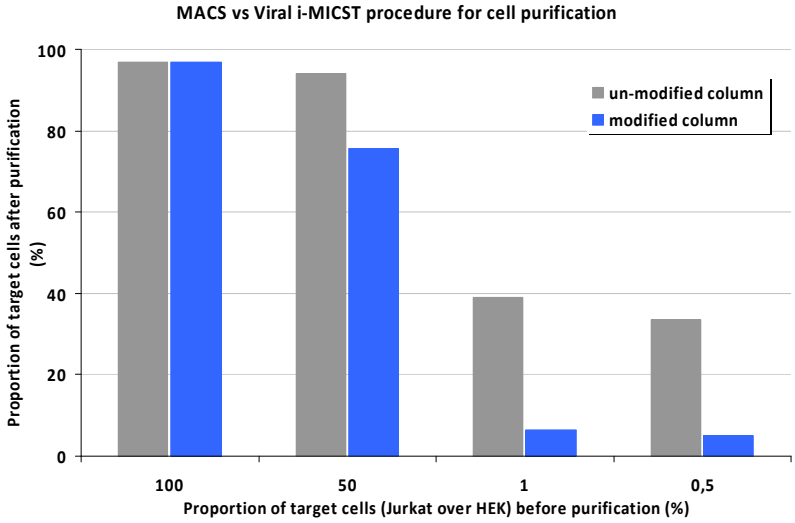


Figure 10: Jurkat were labeled with CD45 microbeads and mixed with different proportions (0, 50, 99 & 99.5%) of HEK-293 cells. 1x10^6 total cells were loaded into either one unmodified or one modified (with Viro-MICST/lentivirus complexes) MACS® LS column. The positive cells selected within the column were then eluted and the proportions of Jurkat cell quantified immediately by flow cytometry after staining with CD3-PE [*****].

These results demonstrated the requirement of using at least two MACS® column when the cell population to purify represents less than 50% of the entire cell number and/or to reach very high purity. This confirms MACS® manufacturer instructions. In the case of Viro-MICST™, the same number of column (1, 2, 3) as usual will be needed, except that the last magnetic column will have to be modified with the Viro-MICST™/viruses complexes.

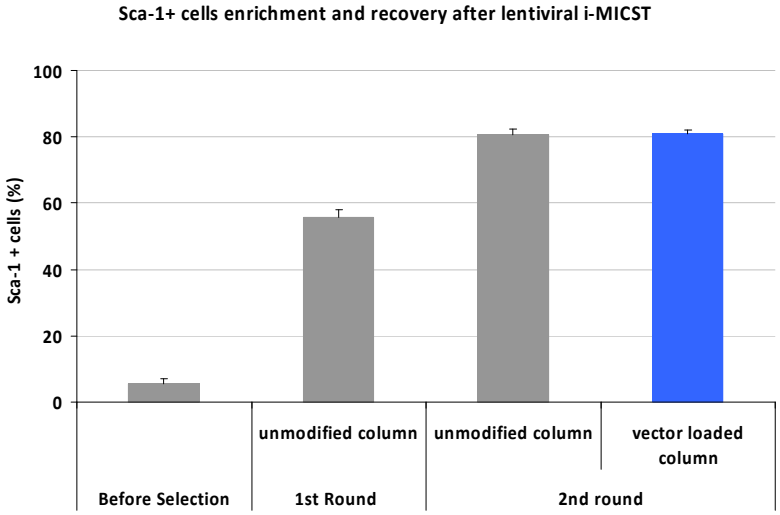


Figure 11: Sca-1+ mouse stem cells were enriched over MACS® LS columns using the Anti-Sca-1 MicroBead Kit (Miltenyi Biotec) and loaded either (*i*) sequentially through two LS columns (MACS procedure) or (*ii*) through one LS column followed by i-MICST™ procedure in the second modified LS column (MOI:3, Viro-MICST:4µL). The selected cells on the column were analyzed for the percentage of anti-Sca-1-FITC positive cells using FACS analysis [*].
These data confirm that treatment of the last MACS® column (LS or MS) with Viro-MICST™/Virus complexes does not interfere with cell sorting.

Efficient capture of virus by Viro-MICST™ reagent

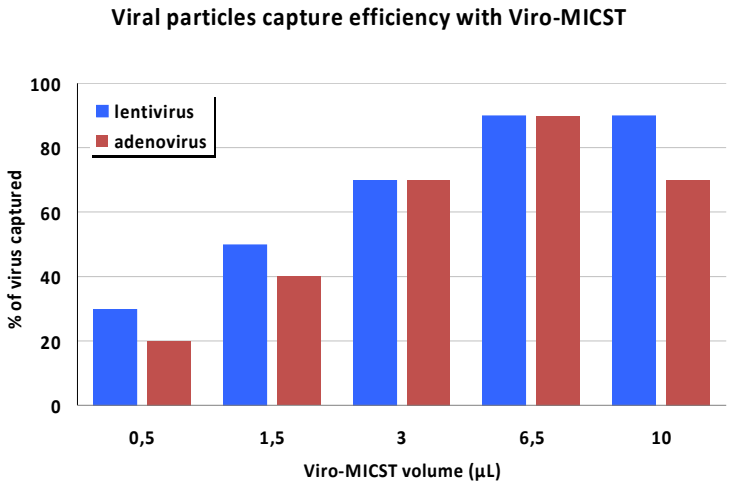


Figure 12: **Lentiviruses** were mixed with different amount of Viro-MICST in RPMI cell culture medium supplemented with 10% FCS and incubated for 20 min to form the complex. The resulting complexes were positioned on top of the 96-Magnet magnetic plate (OZ Biosciences cat # MF10096) for 30 min to sediment the complex. The concentration of the virus in the supernatants was determined using p24 ELISA to quantify the % of virus that were associated with Viro-MICST. **Adenoviruses** were mixed with different amount of Viro-MICST in PBS and incubated for 30 min. The resulting complexes were positioned onto the 96-Magnet magnetic plate for 30 min to sediment the complex. The concentration of virus in the supernatants was determined by infection of COS-7 cells in order to quantify the % of viruses that were associated with Viro-MICST.

Effect of Viro-MICST™ on cell viability and phenotype

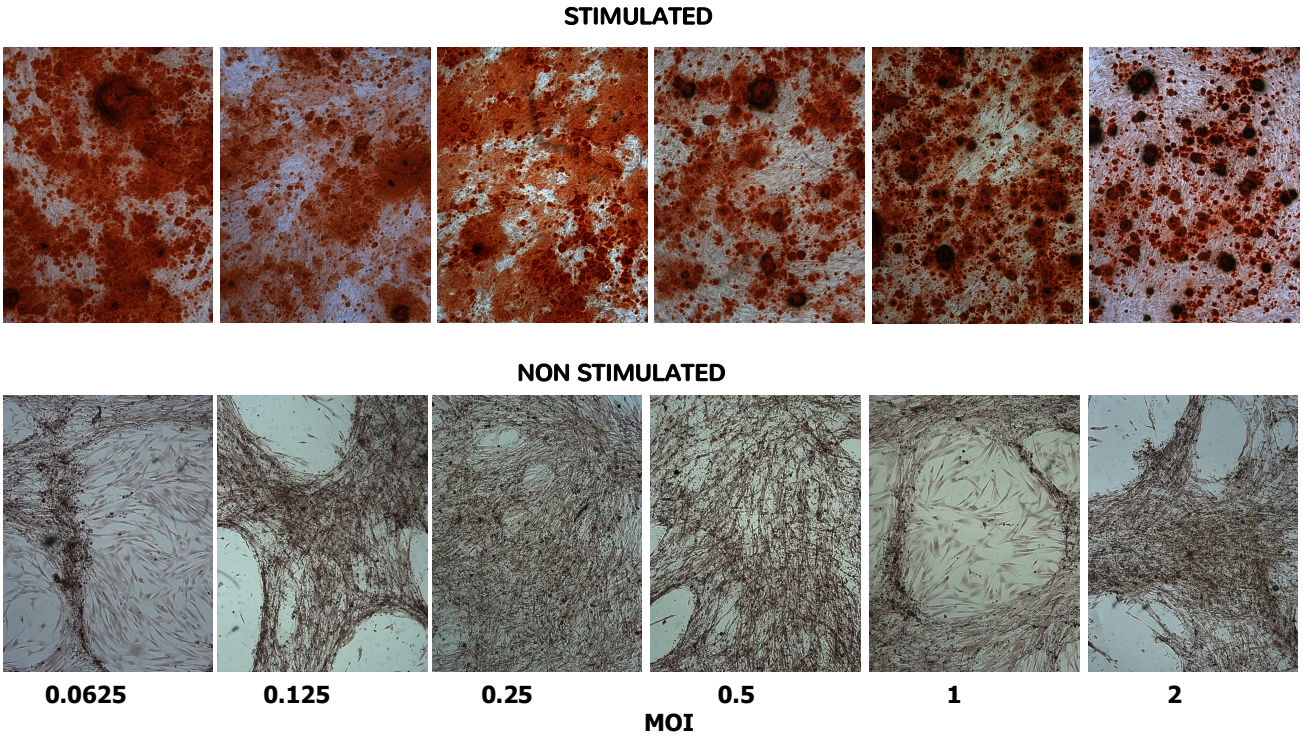


Figure 13: hUC-MSCs maintain their differentiation potential after Viro-MICST™. 1X10^6 hUC-MSCs were labeled with CD105 microbeads and loaded into LS columns modified with Viro-MICST/LV.eGFP (10µL/10^6 VP) at different MOI. Two days after lentiviral i-MICST, the cells were stimulated using an osteogenic medium and 18 days post-stimulation the cells were analyzed using alizarin red staining. Pictures are bright field microscopy images of the stimulated (differentiated) and non-stimulated hUC-MSCs 20 days after viral i-MICST [*].

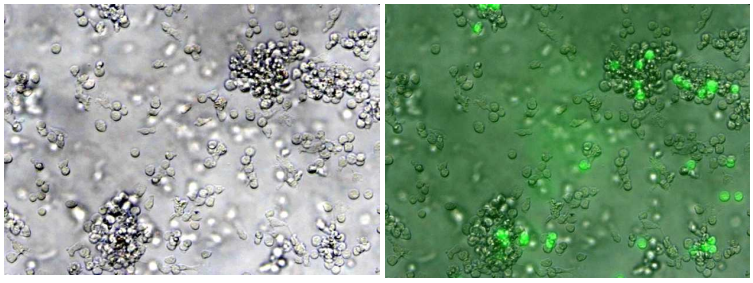


Figure 14: hCB-HSCs maintain their progenitor cell phenotype after lentiviral i-MICST™. hCB-HSCs (1.5 x 10^5 cells/mL) cells were infected at a low cell density without cell stimulation and a MOI of 40. Viral i-MICST procedure: hCB-HSC cells were labeled with CD105 microbeads and loaded into one LS column modified with Viro-MICST/ LV.eGFP (28µL per 10^6 infectious particles). Picture represents the bright field microscopy image of hCB-HSCs differentiated using a colony-forming assay taken 6 days after lentiviral i-MICST. The bars represent 100 m [*].

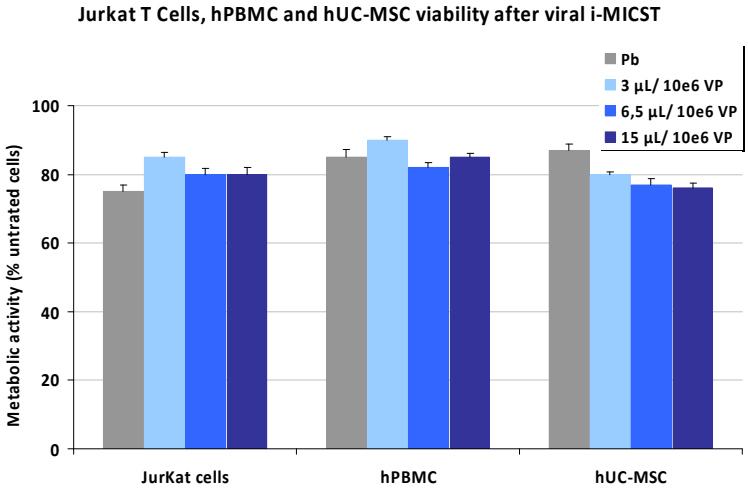


Figure 15: No modification of the metabolic activity after lentiviral i-MICST™. Metabolic activity (MTT) of Jurkat T cells, hPBMCs and hUC-MSCs were performed 48 h after lentiviral i-MICST (MOI = 2, different Viro-MICST/VP ratios) or after polybrene infection (Pb) [*].

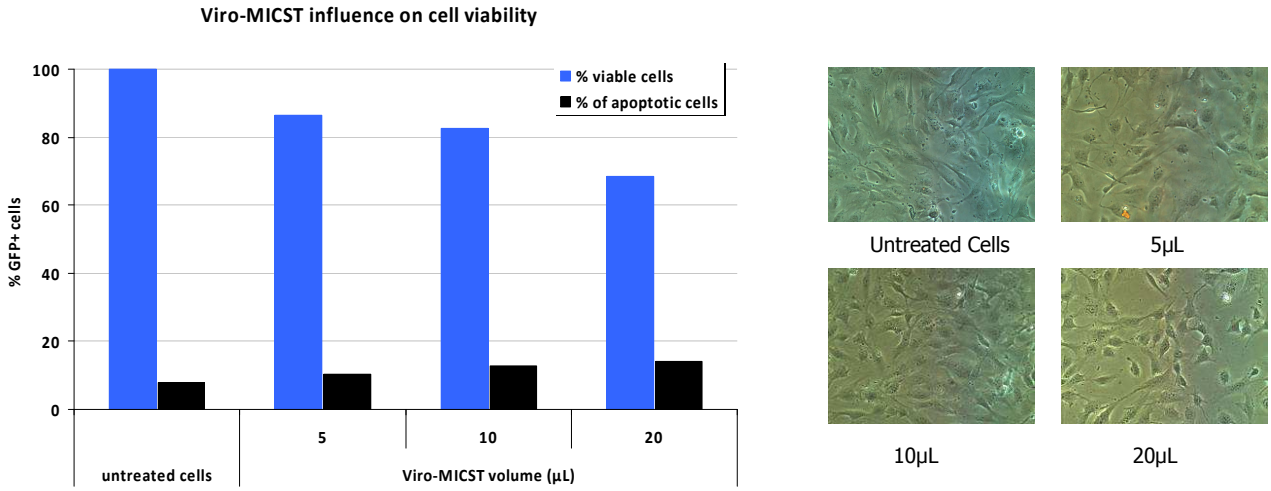


Figure 16: 1X10^6 HMEC-1 cells were labeled with CD105 microbeads and loaded into MS column modified with Viro-MICST/ Ad.eGFP formulated with 5, 10 or 20µL of Viro-MICST per 10^6 ifu and a fixed MOI of 1. The viability was determined by flow cytometry 2 days after transduction using Propidium iodide. Pictures represent HMEC-1 cells 2 days after infection (20x).

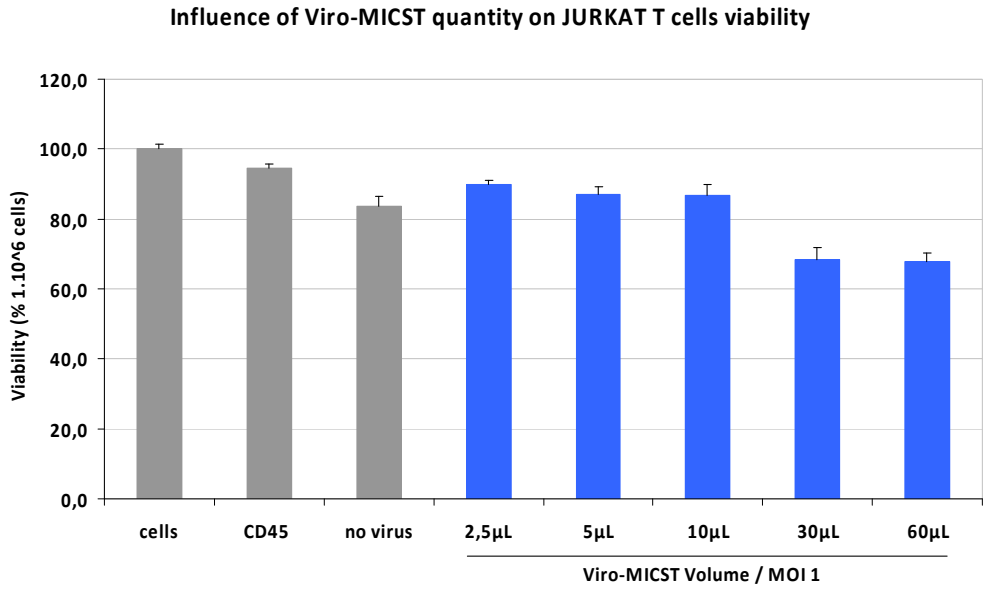


Figure 17: 1x10^6 Jurkat T cells were labelled with CD45 microbeads and loaded into MS column modified with lentivirus (MOI of 1) complexed to several quantities of Viro-MICST. 48H after transduction experiment, viability was assessed using MTT assay. Results were compared to cells that did not receive any treatment. "CD45" and "no virus" bars correspond to cells labelled with CD45 microbeads with no further treatment and to cells were exposed to Viro-MICST reagent alone while sorted onto the column respectively.

The results showed that i-MICST™ procedure does not have any effect on the cell phenotype and viability. If needed, viability could be raised by changing medium 12 to 24 hours after viral i-MICST (data not shown).

Influence of Viro-MICST™, cell number, time course and titers on lentiviral i-MICST™

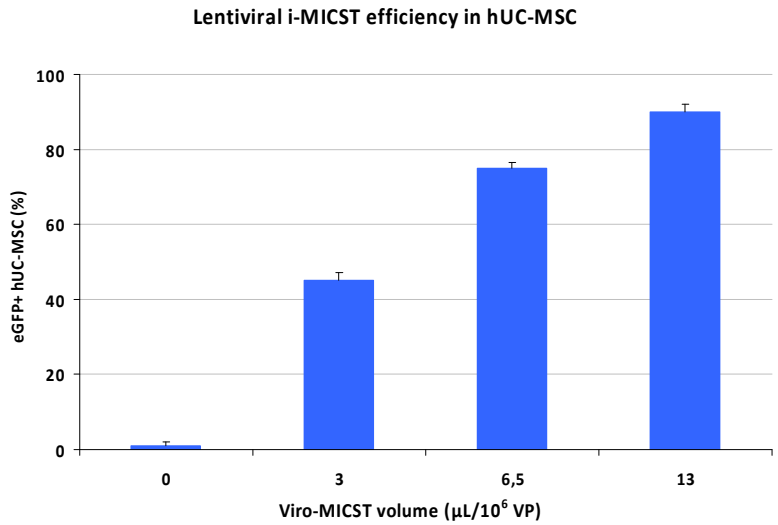


Figure 18: 1X10^6 hUC-MSCs labeled with CD105 microbeads were loaded into LS columns modified with different volume of Viro-MICST complexes to a lentivirus at MOI of 1. Transduction efficiency was measured by FACS 3 days after infection [*].

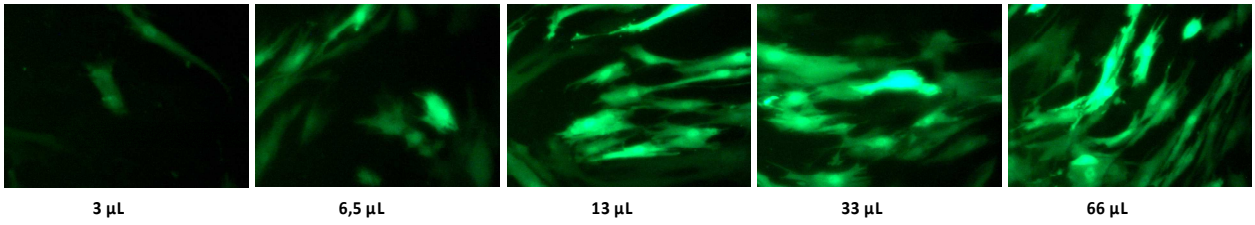


Figure 19: 1x10^6 MSC cells were labeled with CD105 microbeads and loaded into MS columns modified with several quantities of Viro-MICST reagent complexed to lentivirus at a MOI of 1. Transduction efficiency was monitored 72h after transduction by fluorescence imaging.

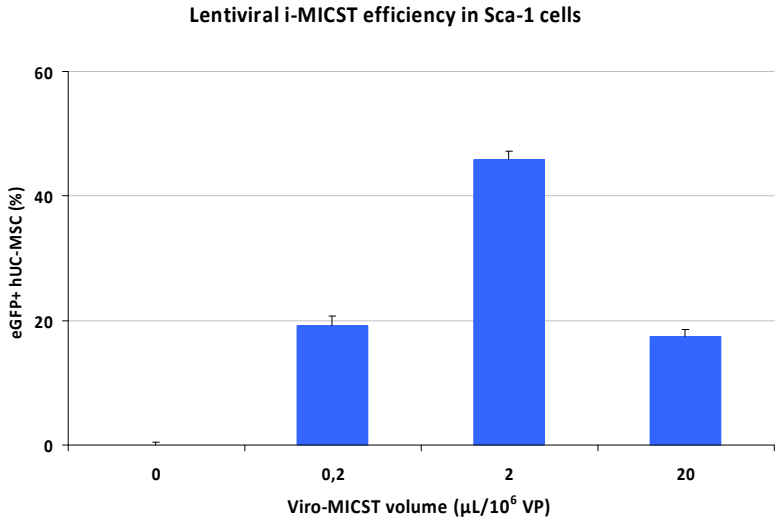


Figure 20: Sca-1+ mouse stem cells were enriched over LS columns using the anti-Sca-1 MicroBead Kit according the manufacturer's protocol (Miltenyi Biotec). Then, 2x10^6 Sca-1+ cells were loaded into LS columns modified with different volume of Viro-MICST™ reagent complexed to a lentivirus at MOI of 3. Transduction efficiency was measured by flow cytometry 6 days after infection [*].

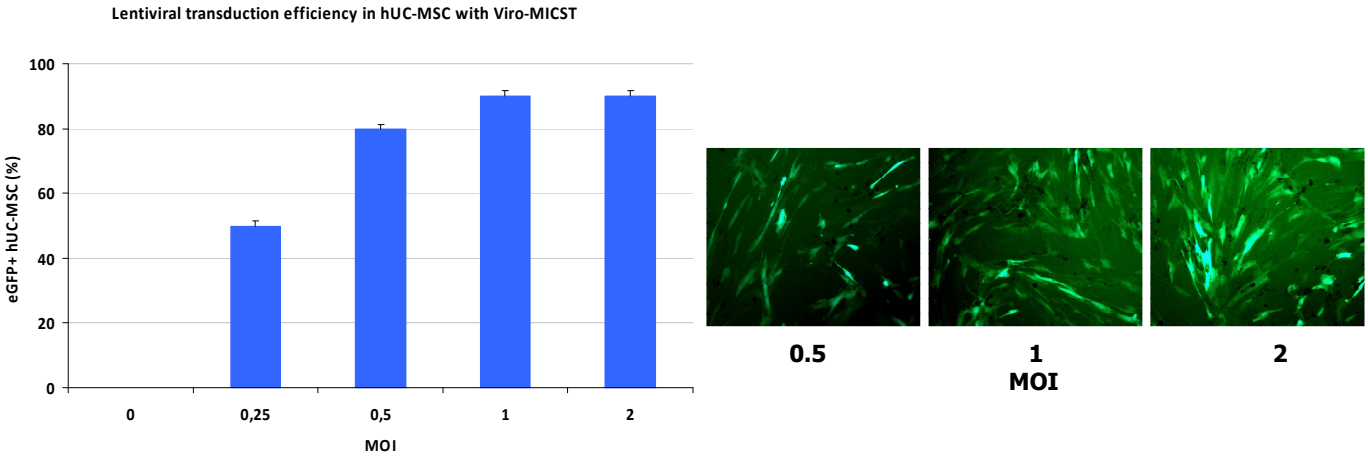


Figure 21: 1X10^6 hUC-MSCs were labeled with CD105 microbeads and loaded into LS columns modified with different MOI and a fixed quantity of Viro-MICST™ reagent (13 µL/10^6 VP). Transduction efficiency was assessed 3 days after transduction by flow cytometry and fluorescent microscopy [*].

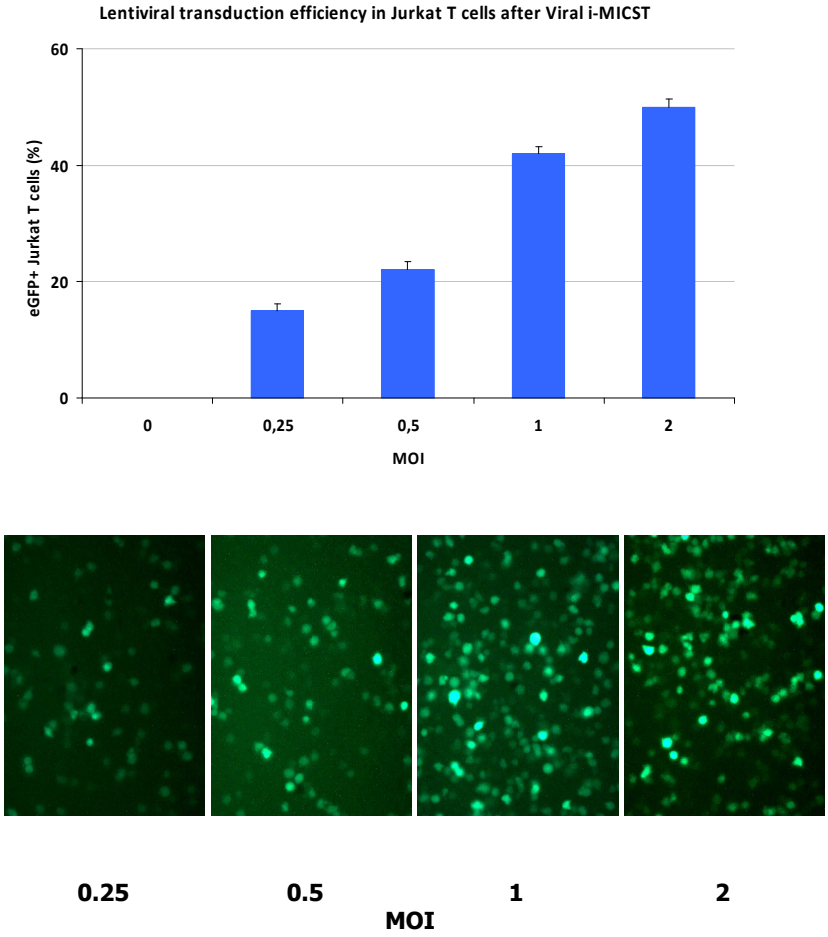
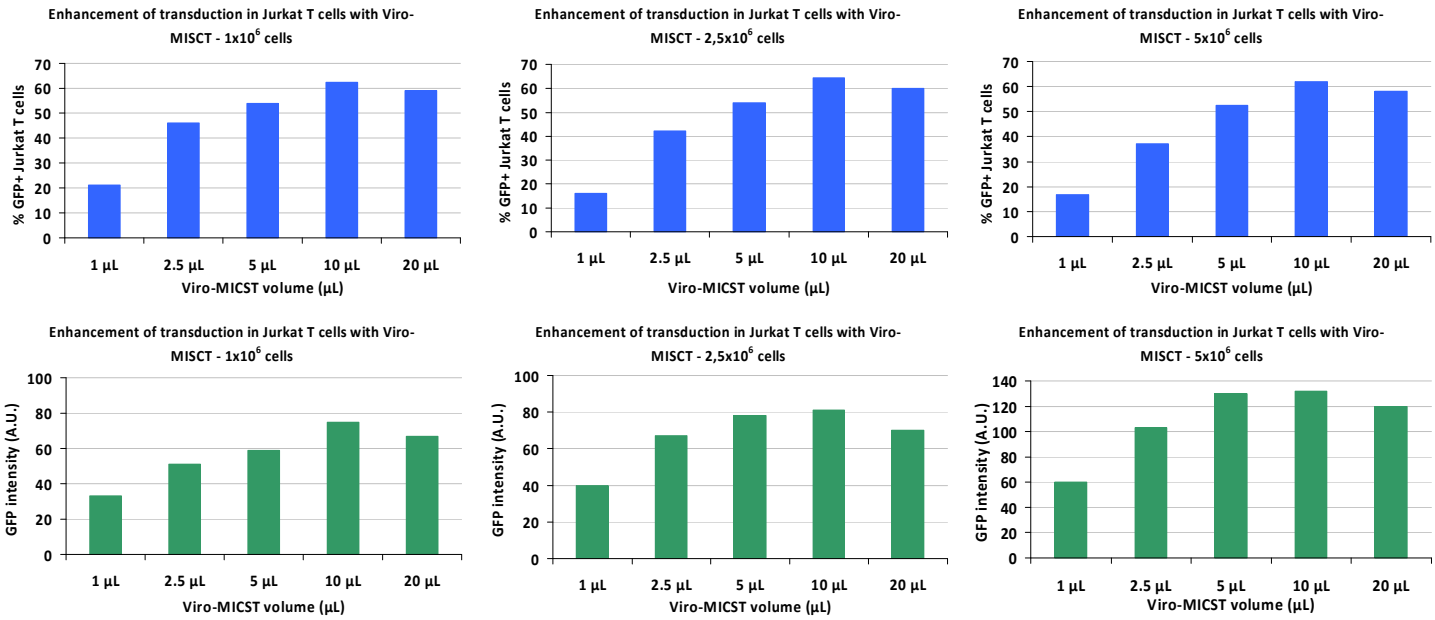
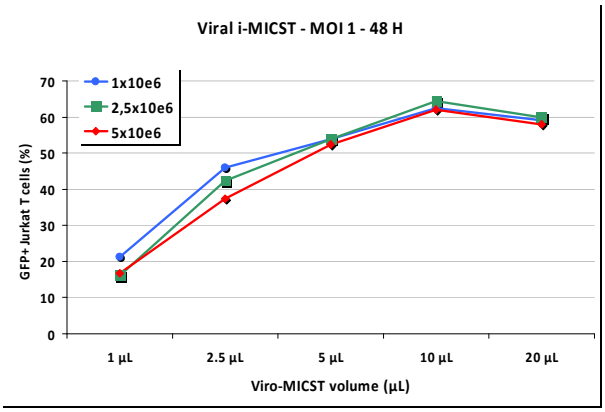


Figure 22: 1X10^6 Jurkat T cells were labeled with CD45 microbeads and loaded into LS columns modified with different MOI and a fixed quantity Viro-MICST (6.5 µL/10^6 VP). Transduction efficiency was assessed 3 days after infection by flow cytometry and fluorescent microscopy [*****].





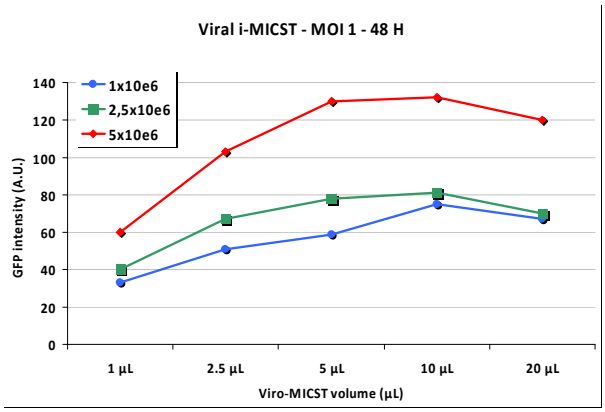
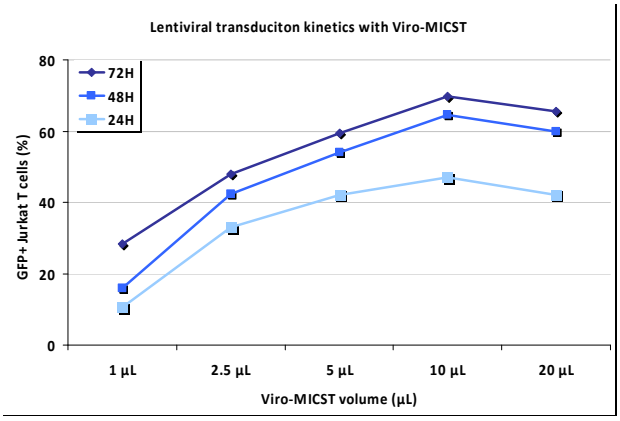


Figure 23: Various numbers of Jurkat T cells were labeled with CD45 microbeads and loaded into MS columns modified with various volumes of Viro-MICST reagent complexed to lentiviral particles at a MOI of 1. Transduction efficiency was assessed 3 days after transduction by FACS.



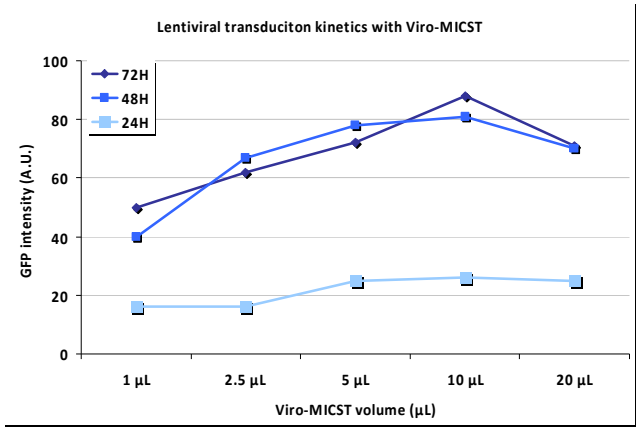


Figure 24: 2,5x10^6 Jurkat T cells were labeled with CD45 microbeads and loaded into MS columns modified with several quantities of Viro-MICST reagent complexed to lentiviral particles corresponding to a MOI of 1. Transduction efficiency was assessed 24, 48 and 72H after transduction by flow cytometry.

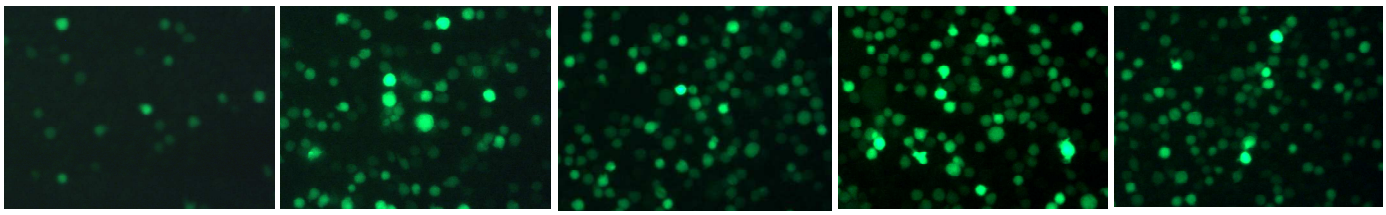


Figure 25: 5x10^6 Jurkat T cells were labeled with CD45 microbeads and loaded into MS columns modified with several quantities of Viro-MICST™ reagent complexed to lentiviral particles corresponding to a MOI of 1. Transduction efficiency was monitored 72H after transduction by fluorescence imaging.

Taking together, the results demonstrated that on the same column format, several numbers of cells can be sorted and transduced at the same time with the same high efficiency when the ratio Viro-MICST/lentivirus is fixed for a given MOI. Moreover, kinetics analysis of GFP showed a regular increase in the % of transduced cells after 24, 48 and 72H.

Influence of Viro-MICST™, virus titers and cell density on adenoviral i-MICST™

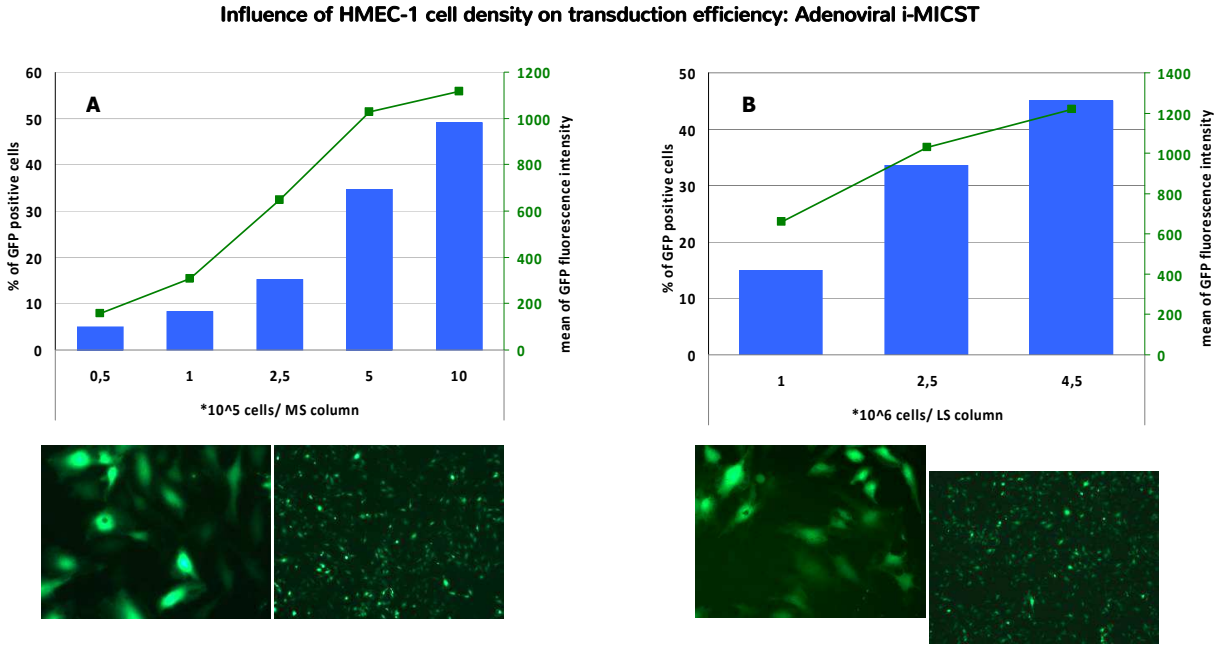


Figure 26A-B: different numbers of HMEC-1 cells were labeled with CD105 microbeads and loaded onto MS (A) or LS (B) column modified with Viro-MICST/ Ad.eGFP formulated with 3µL of Viro-MICST per 10^6 ifu and a MOI of 1. Transduction efficiency was measured by flow cytometry 2 days post-infection. Photos show (4 or 20x magnification) HMEC-1 cells 2 days post-infection at $1X10^6$ cells per MS column or $4.5X10^6$ cells per LS column.

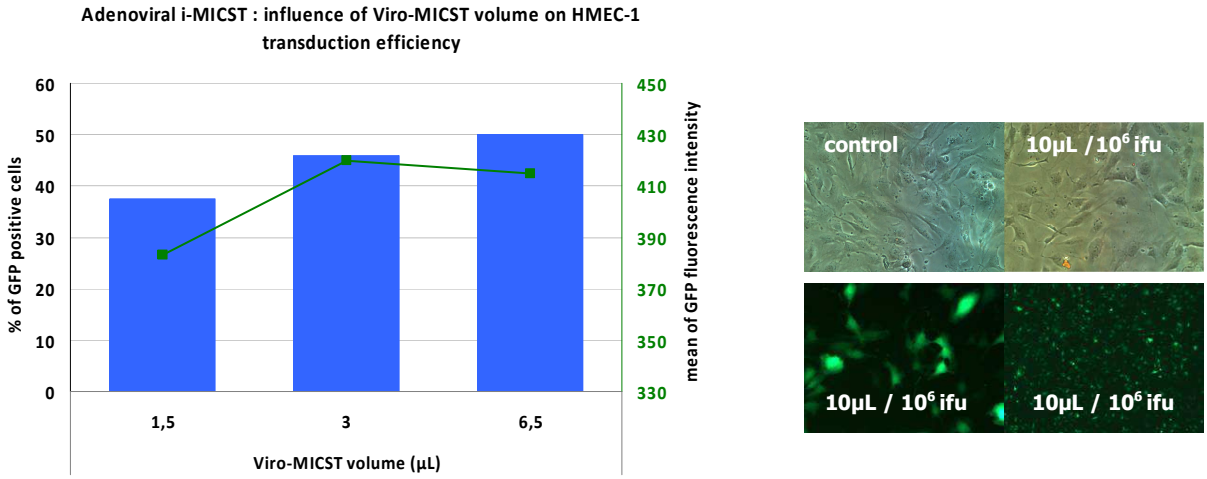


Figure 27: $1X10^6$ HMEC-1 cells were labeled with CD105 microbeads and loaded onto MS column modified with Viro-MICST/ Ad.eGFP formulated with 1.5, 3 or 6.5µL of Viro-MICST per 10^6 ifu and a fixed MOI of 1. Transduction efficiency was measured by flow cytometry 2 days after infection. Pictures show bright field of HMEC-1 cells non transduced (control) and transduced (2 days) using 3µL per 10^6 ifu and a MOI of 1. This enlightens the absence of toxicity. Fluorescent photos (4 or 20x magnification) represent HMEC-1 cells 2 days post-transduction.

Bibliographic references

Please consult our list of references available on the website: www.ozbiosciences.com
***** For more information refer to: Sanchez-Antequera Y *et al.*, Blood. 2011; 117(16):e171-81.

MACS® is a registered trademark owned by Miltenyi Biotec GmbH and the use of MACS® column is proprietary and patented technology. For any further licensed of MACS® system, please contact Miltenyi.