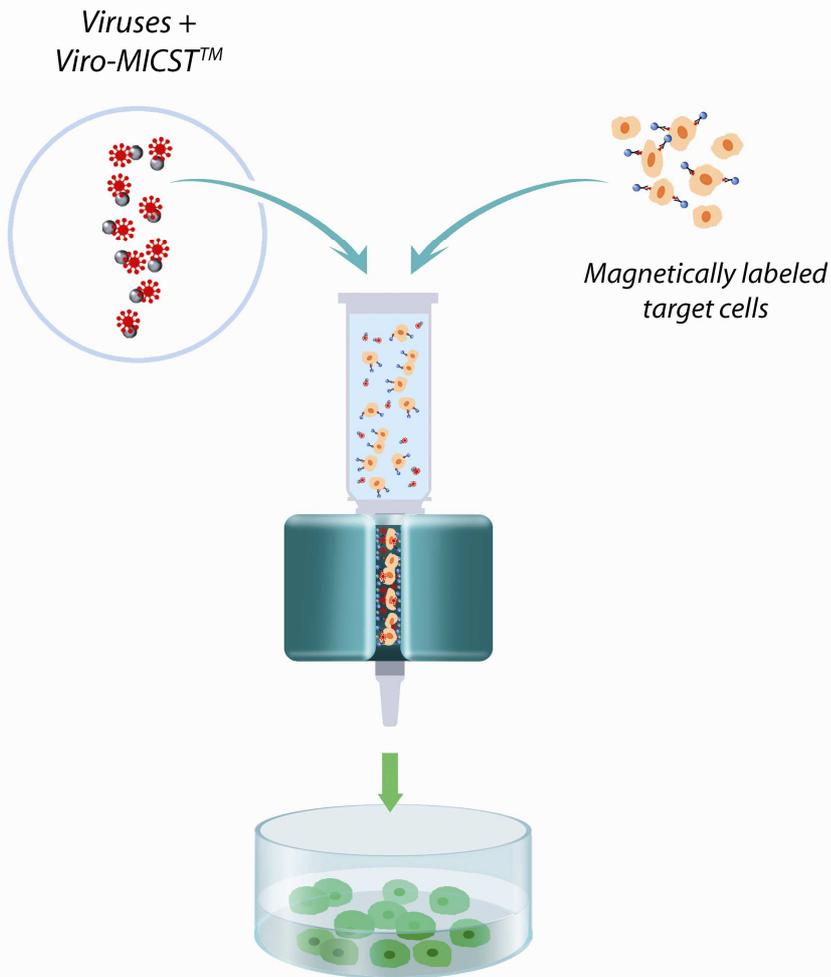


INSTRUCTION MANUAL

*Magnetic Cell Purification &
Transduction in one integrated system*



Instruction Manual

Viro-MICST™ reagent is a new specific magnetic nanoparticles formulation issued from our Magnetofection™ technology. It is specifically designed to achieve high infection rate directly on magnetic cells sorting systems.

Viro-MICST™ allows magnetic cell purification and transduction in one integrated system*

I-MICST™ technology (Integrated **M**agnetic **I**mmuno-cell **S**orting and **T**ransfection/**T**ransduction) is a new platform that allows genetic modifications of cells (transduction or transfection) directly on magnetic cell purification columns or magnetic cell sorting systems. This novel technology combines cell isolation and genetic modification in one simple, efficient and reliable integrated system.

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 cell purification column.

List of Viro-MICST™ Reagents

Catalog Number	Description	Volume (µL)	Number of transductions per small column*	Number of transductions per large column **
VMX250	Viro-MICST™	250	25-50	8-16
VMX500	Viro-MICST™	500	50-100	20-40
VMX1000	Viro-MICST™	1000	100-200	40-80

* Based on MOI of 1 for 10⁶ labeled-cells/column

** Based on MOI of 1 for 2.5x10⁶ labeled-cells/column

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us. For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09 - FRANCE
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117 - USA
Ph : + 1-858-246-7840
Fax : + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com

www.ozbiosciences.com

1. Technology

1.1. Description

Congratulations on your purchase of the **Viro-MICST™** reagent!

I-MICST™ technology (Integrated **M**agnetic **I**mmuno-cell **S**orting and **T**ransfection/**T**ransduction) is a new platform that allows genetic modifications of cells (transduction or transfection) directly on magnetic cell purification columns or magnetic cell sorting systems. This novel 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 using 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.

Viro-MICST™ when combined with magnetic cell sorting technology presents unique properties:

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

1.2. Kit Contents

Kit contents vary according to their size:

- 1 tube containing 0.25 mL of Viro-MICST™ suitable for 25 to 50 assays for 10^6 cells and MOI=1.
- 1 tube containing 0.5 mL of Viro-MICST™ suitable for 50 to 100 assays for 10^6 cells and MOI=1
- 1 tube containing 1 mL of Viro-MICST™ suitable for 100 to 200 assays for 10^6 cells and MOI=1.

Stability and Storage

Storage: +4°C. Viro-MICST™ kits are stable for at least two years at the recommended storage temperature.

- **DO NOT FREEZE THE MAGNETIC NANOPARTICLES!**
- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF MAGNETIC NANOPARTICLES!**

Shipping condition: Room Temperature

2. Applications

2.1. Cell Types

Viro-MICST™ has been successfully tested on a variety of immortalized cell lines and primary cells. Please consult the results and/or our updated list of cells successfully tested available at: 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 frequently updating this list. You can also submit your data to tech@ozbiosciences.com so we can revise this list.

2.2. Virus Types

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

3. General Protocols

3.1. General Considerations

Viro-MICST™ contains magnetic nanoparticles that bind viruses and does not interfere with cell sorting procedure. 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.

Our R&D team has optimized the **Viro-MICST™** reagent in order to provide you with the most straightforward and efficient procedure. Therefore, we recommend you to start by following our general protocol as guidelines. If necessary, we advise you to optimize the experimental condition parameters. Optimal conditions should vary from cell to cell and are highly dependent on the type of virus used, the titers, the composition of the viral solution, and cell culture conditions. Consequently, the amount, concentration and ratio of each individual component (virus and reagent), and the number of cells may have to be adjusted to obtain the best efficiency. Several optimization parameters are available at chapter 3.4.

* 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.

3.2. Key parameters for infection and magnetic enrichment of the target cells

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™ 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 complexes 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.

3.3. Rapid Protocol

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

- The first step 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 1) mainly consists in reaching high purity and simultaneously infecting the target cell population.

3.3.1. Cell Preparation and Pre-enrichment of the target cells

First, purify your cells through one magnetic cell separation column. Please refer to columns manufacturer for target cells pre-enrichment detailed protocol as well as for labeling cells with target antibodies bearing magnetic microbeads. We suggest replacing labeling buffer with complete cell culture medium during this step.

If only one magnetic cell purification column is used (see 3.2) then, label your cells with antibodies coupled magnetic microbeads as indicated by the manufacturer and then go to next step. For the number of cells, refer to Table 1 for each modified MACS® column type.

3.3.2. Viral I-MICST™ procedure

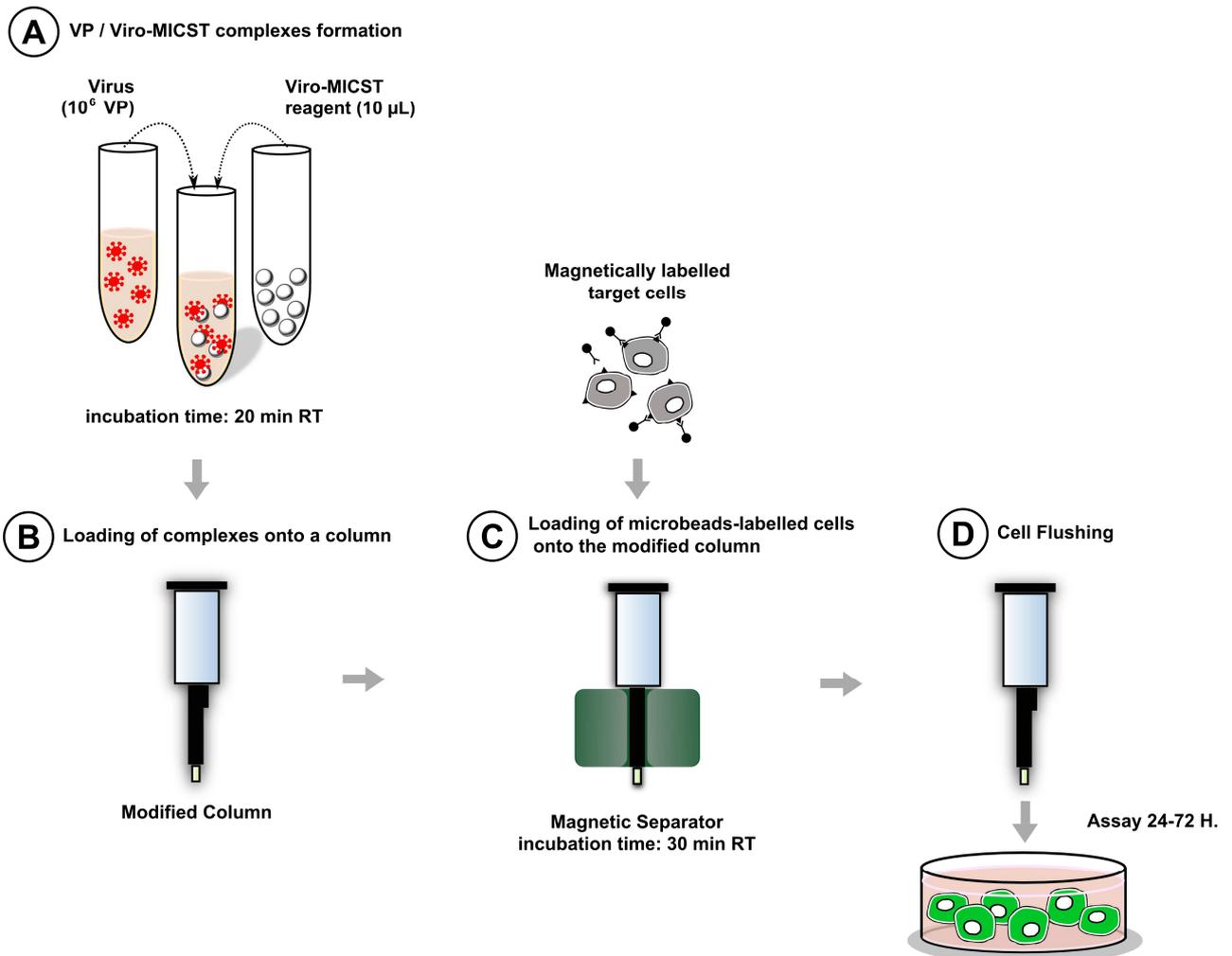


Figure 1. Schematic representation of the Viro-MICST™ procedure. (A) The complexes of virus and Viro-MICST™ reagent are formed and incubated 20 minutes at RT. (B) The complexes are loaded onto the column and allowed to diffuse within the matrix. Then the column is placed into the separator magnet. (C) Cells (magnetically labeled target cells) are loaded into the modified column, washed and then incubated for 30 min onto the magnetic separator. At this step, transduction occurs. (D) Finally, the flushing procedure is performed as described by the manufacturer protocol to recover transduced sorted cells.

A) Viro-MICST™/virus complexes preparation

The recommended volume of **Viro-MICST™** is related to infectious viral particles unit (ifu). The following recommendations can be used as guidelines to quickly achieve very good transduction for 10^6 cells with a MOI of 1. The protocol described below is for a MS column, for the other column formats please refer to Table 1 for the appropriate conditions. As a starting point, we suggest using **10 μ L of Viro-MICST™ for 10^6 infectious particles on a MS-Column.**

- Add **10 μ L of Viro-MICST™** (see table 1) in a 1.5 mL tube.
- Add virus preparation to **Viro-MICST™** and mix immediately by pipetting up and down (do not vortex).
- Adjust the complexes volume to 60 μ L (column void volume) with serum free medium (table 1).
- Incubate 20 min at room temperature

If the complexes volume > 60 μ L then perform a concentration step:

- Add 10 μ L of **Viro-MICST™** in a 1.5 mL tube
- Add virus preparation to **Viro-MICST™** and mix immediately by pipetting up and down (do not vortex).
- Incubate 20 min at room temperature
- Place tube into a Mag-ID device (#DM30000) during 10 min
- Remove supernatant and resuspend pelleted magnetic nanoparticles in 60 μ L of serum free medium.

Note 1: If required to make the proper MOI, virus dilution has to be done in serum-free cell culture medium or other salt-containing buffer (HBS, PBS).

Note 2: Depending on the column size, the complexes volume has to be adjusted to the void volume of the column (table 1).

Table 1: Suggested labeled-cell number, MOI and **Viro-MICST™** conditions.

MACS column	Magnetically labeled cell Number	Infectious particles	Viro-MICST™ Volume (μL)	*Complexes Volume
MS	1×10^6	1×10^6	10	60 μ L
LS	2.5×10^6	2.5×10^6	25	400 μ L
XS	1×10^7	1×10^7	100	6.2 mL

* Complexes volume represents the void volume of MACS® cell separation column. Void volume should be adjusted if working with other manufacturer.

B) Loading complexes onto the cell separation column

- Place a cell separation column into a 15mL tube. Do not position column into magnet at this step
- Load the complexes of **Viro-MICST™** /virus onto the column matrix
- Allow complexes to completely diffuse within the matrix
- Then position the column into the appropriate separator magnet

C) Loading, sorting and transducing cells

While the modified column remains positioned within the separator magnet:

- Load the immuno-magnetically labeled cells and let it infiltrate into the modified column. For the cell number please refer to table 1 or table 5.
- Wash the column with complete cell culture medium (2x1mL). For the washing volume, please refer to the manufacturer of the cell sorting column
- Incubate the column within the separator magnet for 30 min at room temperature

D) Cell flushing and further incubation

- Remove the column from the separator magnet and place it into a new 15 mL tube
- Flush the cells out of the column according to the manufacturer's protocol.
- Incubate the cells at 37°C in a CO₂ incubator under standard conditions until evaluation of the transduction expression.

Note for cell culture: After cell flushing, the cell proliferating rate is also critical and the optimal confluency has to be adjusted. The table 2 shows a suggested number of recovered cells and volume of culture medium in function of the culture dish used.

Table 2: Recommended labeled cell number.

Culture dish	Number of adherent recovered cells	Number of suspension recovered cells	Final culture Volume
96-well	0.5 to 1.5 x 10 ⁴	0.5 to 1 x 10 ⁵	150 µL
24-well	0.5 to 1 x 10 ⁵	2 to 5 x 10 ⁵	500 µL
12-well	1 to 2 x 10 ⁵	2.5 to 10 x 10 ⁵	1 mL
6-well	2 to 5 x 10 ⁵	1 to 2 x 10 ⁶	2 mL
60 mm dish	5 to 10 x 10 ⁵	2.5 to 5 x 10 ⁶	4 mL
90 – 100 mm dish	1.5 to 3 x 10 ⁶	5 to 10 x 10 ⁶	8 mL
T-25 flask	5 to 10 x 10 ⁵	2.5 to 5 x 10 ⁶	5 mL
T-75 flask	2 to 5 x 10 ⁶	5 to 15 x 10 ⁶	10 mL

3.4. Optimization Protocol

In order to get the best out of **Viro-MICST™** reagent, several parameters can be optimized:

- Ratio of **Viro-MICST™** to infectious particles
- Quantity of virus
- Cell number

OZ Biosciences team has investigated numerous factors during the course of the R&D program. Based on our experience, we recommend optimizing one parameter at a time while keeping the other parameters constant.

3.4.1 Ratio of Viro-MICST™ to infectious particles

First, maintain a fixed quantity of infectious particles (we recommend a MOI of 1) and then vary the amount of **Viro-MICST™** over the suggested ranges in the table 3.

Table 3: Suggested ranges of **Viro-MICST™** for optimization with a MOI of 1

MACS column	Magnetically labeled cell Number	Infectious particles	Viro-MICST™ volume (µL)
MS	1 x 10 ⁶	1 x 10 ⁶	2.5 to 20 **
LS	2.5 x 10 ⁶	2.5 x 10 ⁶	6.5 to 50 **
XS	1 x 10 ⁷	1 x 10 ⁷	25 to 200 **

** for sensitive cells (i.e. MSC) do not hesitate to lower Viro-MICST volume. Refer to Viro-MICST results (figure 20) for more details.

3.4.2 Quantity of viral particles

Then once optimal ratio is found, keep it fixed and vary the virus quantity over the suggested range (table 4).

Table 4: Suggested range of virus amounts for optimization.

MACS column	Magnetically labeled cells number	Infectious particles (x10 ⁶)	Viro-MICST™ volume (µL)
MS	1 x 10 ⁶	1 to 20	volume previously determined (3.4.1)
LS	2.5 x 10 ⁶	2.5 to 50	
XS	1 x 10 ⁷	10 to 200	

3.4.3 Cell number

Finally, use the optimized ratio and virus amount obtained previously and vary the cell number to be assayed (table 5).

Table 5: Suggested range of cell number for optimization.

MACS column	Magnetically labeled cells number (x10 ⁶ cells)	Infectious particles (x10 ⁶)	Viro-MICST™ volume (μL)
MS	0.25 to 5	See	See
LS	2.5 to 10	optimisation	optimisation
XS	25 to 100	ratios	ratios

4. Appendix

4.1 Quality Controls

To ensure the performance of each **Viro-MICST™** lot produced, we qualify each component using rigorous standards. The following assays are conducted to qualify the function, quality and activity of each kit.

Specification	Standard Quality Controls
Quality	Size, charge, homogeneity and stability of the Viro-MICST™ reagent controlled by ZetaSizer measurements.
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
Biological Activity	Transduction efficacies on NIH-3T3 and COS-7 cells. Every lot shall have an acceptance specification of > 90% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transduction efficiency	<ol style="list-style-type: none"> Viro-MICST/viral particles (VP) ratio. Optimize the reagent / VP ratio by using a fixed number of VP and vary the amount of Viro-MICST from 2 times less up to 2.5 times more than the suggested amount detailed in the Table 3. Virus quantity. Use different quantities of virus (see Table 4) with the recommended or optimized (above) Viro-MICST/virus ratio. Cell density. A non-optimal cell density at the time of transduction can lead to insufficient uptake. Depending the cells, column clumping can be observed. Optimal cell density is thus difficult to assess: try several densities according to the cells and the column size (Table 5). Virus quality. Virus should be as pure as possible and free of contaminants. Cell condition. 1) Cells that have been in culture for a long time (> 8 weeks) may react differently to transduction. Use freshly thawed cells that have been passaged at least once. 2) Cells should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) alters considerably the transduction efficiency. Cell capture. Ensure that your cells are well immuno-magnetically labeled with the antibody-linked microbeads. A poor or non-specific magnetic labeling will lead to poor capture by magnetic separator. Concentration Step. This step is crucial if Viro-MICST/Virus volume is superior to the column void volume. (1) Optimize the concentration process by increasing the time of capturing onto the MagID device. Tube must stay into the MagID device when supernatant is withdrawn. (2) Ensure total suspension of Viro-MICST/captured virus

	<p>magnetic pellet before loading the column.</p> <p>8. Medium used for preparing Virus/Viro-MICST™ complexes. Depending on the virus type, it may be important to use serum-free medium or buffer (HBS, PBS) during the preparation of the complexes.</p> <p>9. Incubation time. The optimal time range between transduction and assay varies with cells, promoter, expression product, etc. The transduction efficiency can be monitored as soon as one day after experiment. Several reporter genes can be used to quantitatively monitored gene expression kinetics.</p> <p>10. Old Viro-MICST/virus complexes. The complexes must be freshly prepared. Complexes prepared and stored for longer than 1h can be aggregated and should be discarded.</p>
Cellular toxicity	<p>1. Unhealthy cells. 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium conditions (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials, 6) ensure compatibility of labeling magnetic microbeads with cell type.</p> <p>2. Infection/Transgene product is toxic. Use suitable controls such as cells alone, transduction reagent alone or mock infection. A too high MOI can also induce cell death.</p> <p>3. Virus quality - Presence of contaminants. Ensure that virus is pure and contaminant-free.</p> <p>4. Concentration of Viro-MICST/virus is too high. Decrease the amount of Viro-MICST/virus complexes added to the cells by lowering the MOI or the reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously.</p> <p>5. Column Cluttering. Ensure that number of cells is compatible with the separator column used. Large cells can induce a column cluttering that become harmful during the flushing process.</p>

Our dedicated and specialized technical support group will be pleased to answer any of your requests and to help you with your transfection experiments. tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

5. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate (<i>standard size for all cell culture support</i>)
Mega Magnetic plate (<i>mega size to hold 4 culture dishes at one time</i>)
Transfection reagents:
PolyMag Neo (<i>for all nucleic acids</i>)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (<i>for all nucleic acids</i>)
NeuroMag (<i>dedicated for neurons</i>)
SilenceMag (<i>for siRNA application</i>)
Transfection enhancer:
CombiMag (<i>to improve any transfection reagent efficiency</i>)
Viral Transduction enhancers:
ViroMag (<i>to optimize viral transduction</i>)
ViroMag R/L (<i>specific for Retrovirus and Lentivirus</i>)
AdenoMag (<i>for Adenoviruses</i>)
In vivo Magnetofection
In vivo ViroMag (<i>for magnetic assisted viral infection</i>)
In vivo PolyMag (<i>polymer-based magnetic nanoparticles</i>)
In vivo DogtorMag (<i>lipid-based magnetic nanoparticles</i>)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (<i>siRNA transfection reagent</i>)
DreamFect Gold (<i>Transfection reagent for all types of nucleic acids</i>)
VeroFect (<i>for Vero cells</i>)
Ecotransfect (<i>Economical reagent for routine transfection</i>)
FlyFectin (<i>for Insect cells</i>)
i-MICST TECHNOLOGY
Viro-MICST (<i>to transduce directly on magnetic cell purification columns</i>)
3D TRANSFECTION TECHNOLOGY
3DfectIN (<i>for hydrogels culture</i>)
3Dfect (<i>for scaffolds culture</i>)
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (<i>for High Yield Protein Expression</i>)
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN (<i>delivery reagent for antibodies</i>)
Pro-DeliverIN (<i>delivery reagent for protein in vivo and in vitro</i>)
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit
MTT cell proliferation kit
β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K ⁺ and Na ⁺ 1g
G-418, Sulfate 1g
X-Gal powder 1g

Our dedicated and specialized technical support group will be pleased to answer any of your request and to assist you in your experiments. Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list. <http://www.ozbiosciences.com>.

Purchaser Notification

Limited License

The purchase of the Viro-MICST™ Reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the transduction of cells as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the Viro-MICST™ Reagent. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

Buyers may end this License at any time by returning all Viro-MICST™ Reagent material and documentation to OZ Biosciences, or by destroying all Viro-MICST™ components. Purchasers are advised to contact OZ Biosciences with the notification that a Viro-MICST™ kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

This document covers entirely the terms of the Viro-MICST™ Reagent research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The Viro-MICST™ Reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Business Development
OZ Biosciences SAS
Parc Scientifique de Luminy
Zone Luminy Entreprise
163, avenue de Luminy – Case 922
13288 Marseille Cedex 9 - FRANCE
Ph: +33 (0)4. .86.94.85.16
Fax: +33 (0)4. .86.94.85.15
E-mail: business@ozbiosciences.com

CONTACTS

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
FRANCE

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117
USA

Ph : + 1-858-246-7840
Fax : + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com