Viral Applications

Viro-MICST™

Efficient and specific target cells transduction

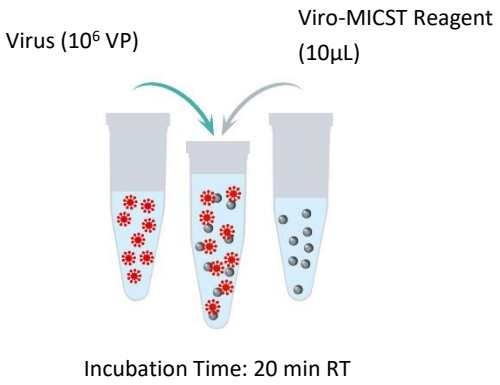
Protocol

OZBIOSCIENCES
The art of delivery systems

Viro-MICST – Quick Protocol

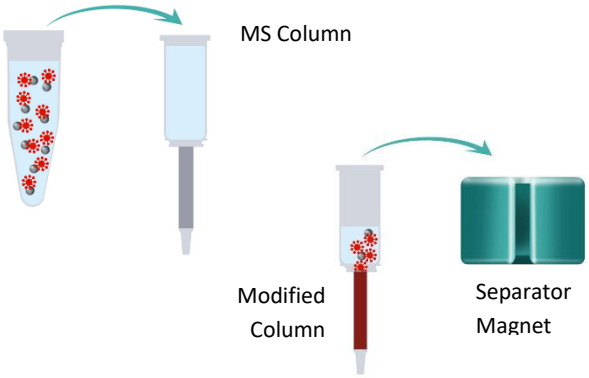
Viro-MICST/Virus Complexes Formation

- Add 10µL of Viro-MICST in a 1.5 mL tube
- Add Virus suspension to Viro-MICST
- Mix immediately by pipetting
- Incubate 20 min at Room Temperature
  (for volume >60 µL refer to instruction manual)



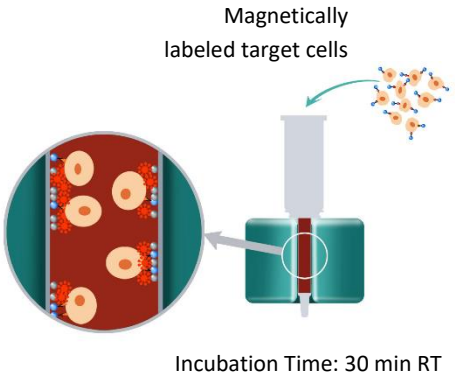
VMS Column Loading with Complexes

- Place a MS column in a 15 ml tube
  (do not position column onto magnet)
- Load complexes of Viro-MICST/VP onto column
- Mallow complexes to diffuse within the matrix
- Position the column into separator magnet



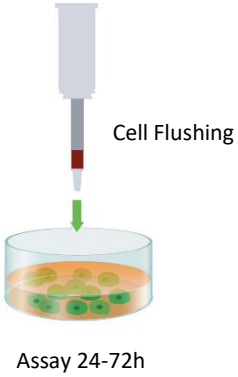
Loading/Sorting/Transducing Target Cells

- Load magnetically labeled cells into the modified column
- Wash column with complete medium
- Incubate column for 30 min onto the magnetic separator
  (refer to column manufacturer for magnetic cell labelling)



Cell flushing and further incubation

- Remove the column from the magnetic separator and place it into a new 15 mL tube
- Flush the cells
- Incubate cells under standard culture conditions until assay evaluation



This quick protocol is suitable for $1x10^6$ cells on a MS column (MACS® column) with a MOI of 1*. Please consult the full instruction manual for detailed information

IMPORTANT NOTES – Before you begin

1. This short protocol is suitable for transducing 10^6 cells on a MACS® MS column** with a MOI of 1.

2. Viro-MICST does not interfere with magnetic cell sorting devices and we recommend following rigorously the cell sorting protocol given by the manufacturer.

3. High yield of purification is reached when using at least two columns; apply Viro-MICST always on the last column of the process.

4. Two magnetic columns should be used if:
   - Cell population to be purified represents less than 50% of the total cell population
   - The degree of purity needed is > 90%.

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

   - Cell preparation and Pre-enrichment of the target cells: Magnetically label your cells following the manufacturer's instructions.

NOTE: OZ Biosciences does not provide magnetic cell separation systems, please refer to the manufacturer instructions protocols for this step.

   - Viro-MICST™ procedure: infect and transduce target cells during the purification process.

MACS Column**	Magnetically labeled cell number	Infectious particles	Viro-MICST™ volume	***Complexes volume
MS	1 x 10^6	1 x 10^6	10 µL	60 µL
LS	2.5 x 10^6	2.5 x 10^6	25 µL	400 µL
XS	1 x 10^7	1 x 10^7	100 µL	6.2 mL

Table 1: Suggested labeled-cell number, MOI and Viro-MICST™ conditions depending on the size of separation column.

**** complexes volume represents the void volume of MACS* cell separation column*

IMPORTANT NOTES

- Do not freeze the magnetic nanoparticles!
- Polybrene or other additives must NOT be used in combination with Viro-MICST

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

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***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.*

ViroMICST Reagent | Specifications

Package content	VMX250: 250 µL of Viro-MICST™ VMX500: 500 µL of Viro-MICST™ VMX1000: 1mL of Viro-MICST™
Shipping conditions	Room Temperature
Storage conditions	Store the Viro-MICST™ reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled.
Product description	Viro-MICST™ allows magnetic cell purification and transduction in one integrated system
Important notice	For research use only. Not for use in diagnostic procedures.

1. Cells preparation and pre-enrichment of 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 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.

2. 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 µL of Viro-MICST™ for 10^6 infectious particles on a MS-Column.**

- Add **10 µL of Viro-MICST™** (refer to 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 µL (column void volume) with serum free medium (refer to table 1).
- Incubate 20 min at room temperature

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

- Add 10 µL 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 µL of serum free medium.

Notes:

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

3. 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

4. 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

5. 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 CO_2 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.

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
6-well	2 to 5 x 10^5	1 to 2 x 10^6	2 mL

Table 2: Recommended labeled cell number.

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

We recommend optimizing one parameter at a time while keeping the other parameters constant.

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.

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

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

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

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).

MACS column	Magnetically labeled cell Number	Infectious particles($x10^6$)	Viro-MICST™ volume (µL)
MS	1×10^6	1 to 20	volume previously determined
LS	2.5×10^6	2.5 to 50	
XS	1×10^7	10 to 200	

Table 4: Suggested range of virus amounts for optimization.

3. Cell number

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

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

Table 5: Suggested range of cell number for optimization

Additional products for Viral Transduction Enhancement

- **ViroMag RL** for enhancing Lentiviral and Retroviral transduction efficiency
- **AdenoMag** specific for Adenoviral and AAV transduction

Additional products for Virus Capture and Concentration

- **Mag4C-LV** for Lentiviruses
- **Mag4C-AD** for Adenoviruses

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 this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transduction of cells with viruses 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 us at OZ Biosciences. Buyers may end this License at any time by returning all Viro-MICST reagents 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 reagent 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 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
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.

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