Viral Applications

# ViroMICST Stem

Transduction enhancer

Stem cells infection during magnetic cell purification

# Protocol

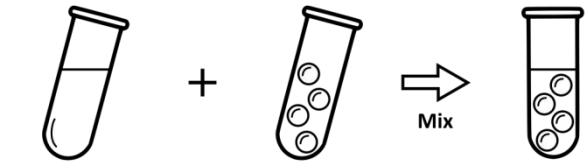
OZBIOSCIENCES
The art of delivery systems

# Quick proto schema for infection/transduction on separation column

The following protocol is given for MS column with a void volume of 60 µL; adjust or adapt volumes depending on the column format. If the lentiviral transduction/infection conditions are unknown, we recommend starting with a MOI of 2 using a lentiviral vector encoding for a fluorescent protein and 3 different volumes of ViroMICST Stem Reagent ST with 3 µL of Reagent VM.

## ViroMICST STEM Working Solution

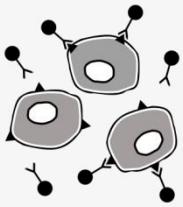
Before Beginning, prepare 3 ViroMICST Stem Working Solutions (WS) by mixing 2, 5 or 10 µL Reagent ST with 3µL Reagent VM



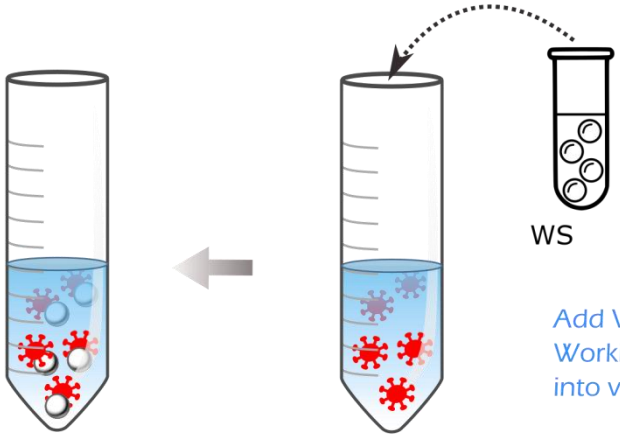

Reagent ST 2, 5 and 10 µL | Reagent VM 3µL | ViroMICST Stem Working Solution

| | ViroMICST Stem working solution | |
|---|---|---|
| | Reagent ST | Reagent VM |
| Tube 1 | - | - |
| Tube 2 | 2 µL | 3 µL |
| Tube 3 | 5 µL | 3 µL |
| Tube 4 | 10 µL | 3 µL |

Magnetically label cells, refer to instruction manual



1a MAGNETICALLY LABELLING OF TARGET CELL POPULATION

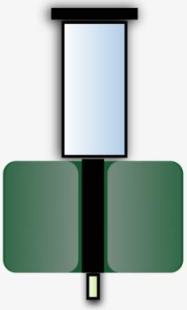



Add ViroMICST STEM Working Solution into viral suspension

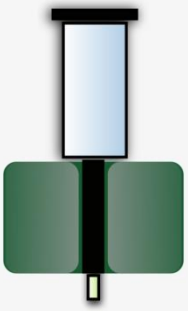
Incubation time: 25 min.

1b COMPLEXES FORMATION

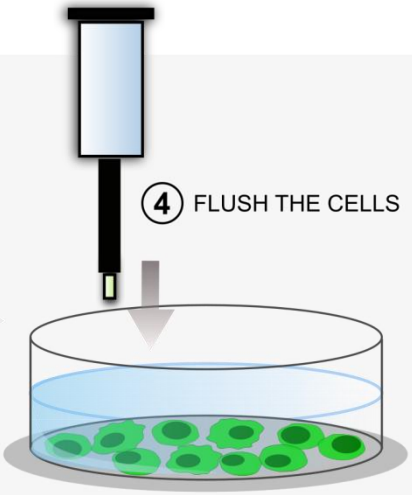
Refer to instruction manual



2 LOAD CELLS ON COLUMN



3 LOAD COMPLEXES ON COLUMN
Incubation time: 30 min.




5 ASSAY (24/96H)



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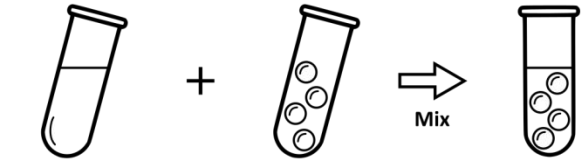
# Quick proto schema for infection/transduction on column-free separation magnet

The following protocol is given for cell sorting using column-free immunomagnetic separation magnet in 2.5 - 5 mL volume; adjust or adapt volumes depending on cell sorting apparatus format or cell culture volume. If the lentiviral transduction/infection conditions are unknown, we recommend starting with a MOI of 2 using a lentiviral vector encoding for a fluorescent protein and 3 different volumes of ViroMICST Stem Reagent ST with 3 µL of Reagent VM

## ViroMICST STEM Working Solution

Before Beginning, prepare 3 ViroMICST Stem Working Solutions (WS) by mixing 2, 5 or 10 µL Reagent ST with 3µL Reagent VM



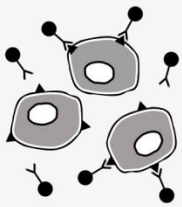

Reagent ST 2, 5 and 10 µL

Reagent VM 3µL

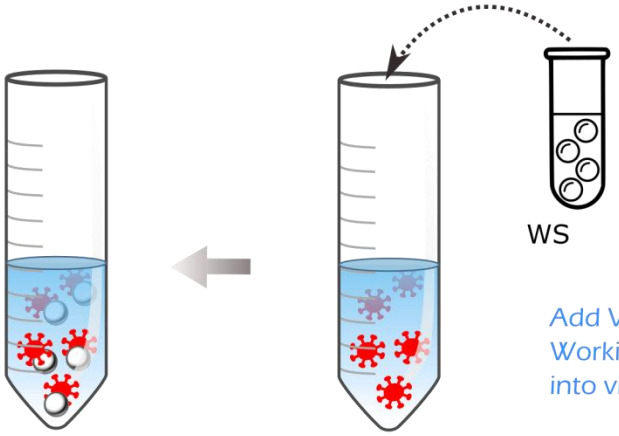
ViroMICST Stem Working Solution

| | ViroMICST Stem working solution | |
|---|---|---|
| | Reagent ST | Reagent VM |
| Tube 1 | - | - |
| Tube 2 | 2 µL | 3 µL |
| Tube 3 | 5 µL | 3 µL |
| Tube 4 | 10 µL | 3 µL |

Magnetically label cells, refer to instruction manual



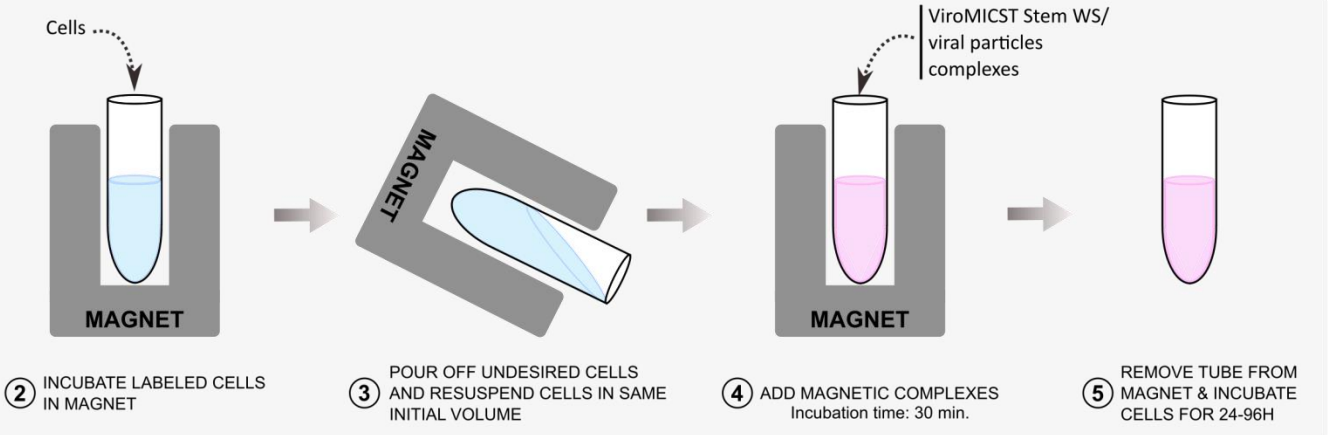
1a MAGNETICALLY LABELLING OF TARGET CELL POPULATION




Add ViroMICST STEM Working Solution into viral suspension

Incubation time: 25 min.

1b COMPLEXES FORMATION




2 INCUBATE LABELED CELLS IN MAGNET

3 POUR OFF UNDESIRED CELLS AND RESUSPEND CELLS IN SAME INITIAL VOLUME

4 ADD MAGNETIC COMPLEXES Incubation time: 30 min.

5 REMOVE TUBE FROM MAGNET & INCUBATE CELLS FOR 24-96H

# IMPORTANT NOTES – Before you begin

- ✓ This short protocol is suitable for transducing 0.1 to 1x$10^7$ labeled-cells per immunomagnetic cell separation (magnet or column)
- ✓ Viro-MICST Stem does not interfere with magnetic cell sorting devices and we recommend following rigorously the cell sorting protocol given by the manufacturer.
- ✓ Using column purification, high yield of purification is reached when using at least two columns; apply Viro-MICST always on the last one of the process.
- ✓ Allow reagents to reach room temperature before starting
- ✓ Do not change your protocol for cell sorting after immunomagnetic labelling, simply add complexes of viral particles/ViroMICST Stem to the cell sorting apparatus (column or magnet).
- ✓ Do not mix Reagent ST or Reagent VM with any other viral enhancer or chemicals
- ✓ If transduction conditions are unknown, we recommend beginning with a MOI of 2
- ✓ We suggest starting with 2, 5 and 10 µL of ViroMICST STem Reagent ST with 3µL Reagent VM – on the basis of MS column size from Miltenyi or 2.5 to 5 mL volumes for cell sorting magnet
- ✓ Refer to optimization protocol for gaining more information on how to optimize conditions.

OZ Biosciences does not provide magnetic cell separation systems ; OZ Biosciences does not provide the immunomagnetic beads. We recommend following rigorously the cell sorting protocols for both items given by the manufacturers.

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

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

| | |
|---|---|
| Package content | VMXS100: ViroMICST Stem 100 transductions (500 µL of Reagent ST + 300 µL of Reagent VM)<br>VMXS300: ViroMICST Stem 300 transductions (1500 µL of Reagent ST + 900 µL of Reagent VM) |
| Shipping condition | Room Temperature |
| Storage conditions | Upon reception and for long-term use, store Reagent ST at -20°C and Reagent VM at +4°C. |
| Shelf life | 1 year from the date of purchase when properly stored and handled |
| Product description | Viro-MICST Stem Transduction Enhancer was specifically designed for **stem cell lentiviral transduction** in order to achieve high infection rate directly on **magnetic cells sorting devices** (column or magnet) |
| Important notice | For research use only. Not for use in diagnostic procedures |

## 1. Cells Preparation

Refer to columns manufacturer for target cells pre-enrichment detailed protocol as well as for labelling cells with nano-sized or micro-sized immunomagnetic beads.

## 2. Optimization Protocol

As a starting point, we recommend trying 3 different volumes of Reagent ST with 3 µL of Reagent VM ; however several parameters can be optimized such as : MOI, volumes of each tube composing ViroMICST Stem per column, cell number…
It is generally suggested to optimize one parameter at a time while keeping the other parameters constant.

a. Optimizing ViroMICST Stem volume – example for 0.1 to 1x$10^7$ labeled-cells per immunomagnetic cell separation (magnet or column) : using a fixed MOI, vary the volume of each tubes composing ViroMICST Stem working solution.

NOTE : These conditions are given for MS column or using a separation magnet for up to 5 mL cell suspension. Adapt volumes according to other cell sorting columns format or separation volumes.

b. Quantity of viral particles: once the ideal ratio of ViroMICST Stem is found, keep it fixed and vary MOI.

c. Cell number: finally use the best ratio and MOI found in (a) and (b) and vary the cell number to be assayed.

# NOTES

Additional products for Viral Transduction Enhancement

- **ViroMag** for enhancing viral transduction efficiency (suitable for all viruses)
- **ViroMag R/L** for enhancing Lentiviral and Retroviral transduction efficiency
- **AdenoMag** specific for Adenoviral and AAV transduction

Additional products for Virus Production

- **Helix-IN DNA Transfection Reagent**

Additional products for Virus Capture and Concentration

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

**Purchaser Notification**

**Limited License**
The purchase of the ViroMICST Stem 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) for the sole purpose of in-house research only, provided that no license, right or permission is granted hereunder to a non-academic, for-profit or commercial Licensee to use the ViroMICST Stem reagent for *ex vivo* gene therapy for hemoglobinopathies. The license does not include the use for any commercial or development purpose, including but not limited to any use for a) manufacturing, production, quality control, b) providing services, information or data, c) therapeutic, diagnostic, vaccine or prophylactic purposes or d) any applications which require regulatory approval as well as e) any clinical activities *in vivo* or *ex vivo*. The licensed use is limited to transfection of nucleic acids 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.
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**Product Use Limitations**
The ViroMICST Stem 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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