Miltenyi Biotec

StemMACS™ mRNA Transfection Kits

Contents

1. Description

This product is for research use only.

Products StemMACS mRNA Transfection Kits

Order no.	Components
130-132-978	125 µL StemMACS Transfection Reagent 25 mL StemMACS Transfection Buffer
130-132-949	250 µL StemMACS Transfection Reagent 25 mL StemMACS Transfection Buffer
130-104-463	0.5 mL StemMACS Transfection Reagent 25 mL StemMACS Transfection Buffer

Quality control Functionally tested for transfection of human fibroblasts.

Storage Store all components at 2–8°C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The transient, non-integrative expression of key developmental regulators, recombinases, or markers via mRNA transfection is a powerful tool for modulating cell fate. The StemMACS mRNA Transfection Kit is a novel lipid-based transfection system that has been designed for efficient mRNA delivery into various cell types. StemMACS mRNA Transfection Reagent has been selected for its minimal cytotoxicity and high transfection efficiency. It is therefore particularly suited for sensitive cell lines such as pluripotent stem cells and complex transfection schedules that involve repeated mRNA delivery over several days. The StemMACS mRNA Transfection Kit has been successfully used for transfection of primary human fibroblasts and the generation of iPS cell lines by mRNA reprogramming.

For successful transfection, use a protocol that is optimized for the cell type of interest. StemMACS eGFP mRNA or StemMACS Nuclear eGFP mRNA allow an easy read-out for transfection efficiency and are recommended as positive controls when establishing new transfection protocols. Critical parameters for successful mRNA transfection include mRNA quality and effective suppression of the innate antiviral response to single-stranded RNA. It is recommended to use only high quality mRNA preparations that have been validated for mRNA transfection and incorporate modified nucleotides such as pseudouridine and 5-methylcytidine (e.g. StemMACS mRNA products). Depending on the application and cell line, supplementation of the cell culture medium with B18R protein may be necessary to ensure complete suppression of the innate antiviral response during transfection.

1.2 Applications

- Transient, non-integrative delivery of mRNA encoded factors into a broad range of cell types, including primary fibroblasts and human iPS cells
- mRNA reprogramming of human fibroblasts
- mRNA-induced differentiation of stem and progenitor cells
- mRNA-induced transdifferentiation of differentiated cells
- mRNA-induced recombination
- Transient labeling with fluorescent proteins

2. Protocol

▲ RNA is susceptible to degradation by exogenous ribonucleases. Wear gloves and use RNase-free tubes, reagents, and pipettes. It is recommended to use low RNA/DNA-binding tubes and barrier pipette tips when handling mRNA.

1. The day before transfection, seed cells in 24-well or 6-well tissue culture plates. For recommended seeding conditions refer to table 1.

   ▲ **Note:** The transfection can be carried out in the presence of antibiotics (e.g. penicillin, streptomycin) and serum. A medium change prior to transfection is not required.

   Table 1: Recommended seeding conditions

	per 24-well plate	per 6-well plate
Recommended seeding density	1–5×10^4 cells	10^4–10^5 cells
Cell culture medium	475 µL	1400 µL
mRNA transfection complex (added the following day)	25 µL	100 µL
Total volume	**500 µL**	**1500 µL**

2. On the day of transfection, thaw mRNA aliquots slowly on ice. Prepare two sterile, RNAse-free microfuge tubes for each well to be transfected.
3. Dilute the mRNA in StemMACS Transfection Buffer. Mix by pipetting up and down.

   ▲ **Note:** The amount of mRNA needed may vary with the cell type, the protein encoded by the mRNA, and the specific requirements of the downstream application. Initially, it is recommended to test at least three different mRNA concentrations in the range of 0.1 to 3 µg mRNA to find the optimal amount for transfection. Refer to table 2 for guidelines on setting up the first experiment.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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4. Dilute StemMACS Transfection Reagent in StemMACS Transfection Buffer. Mix by pipetting up and down.

   ▲ **Note:** In most cases, using a ratio of 1:3 (w/v) of mRNA to StemMACS Transfection Reagent will be appropriate, i.e., use 3 µL of StemMACS Transfection Reagent for each 1 µg of mRNA to be transfected. The volumes given in table 2 are based on this recommended ratio. In case further optimization is needed this ratio may be varied in the range between 1:2 to 1:4 (w/v).

Table 2: Guidelines for preparation of the mRNA transfection complex

24-well format

Tube 1		Tube 2	
mRNA	**StemMACS Transfection Buffer**	**StemMACS Transfection Reagent**	**StemMACS Transfection Buffer**
150 ng	Adjust to 12.5 µL with buffer	0.45 µL	12.15 µL
250 ng	Adjust to 12.5 µL with buffer	0.75 µL	11.75 µL
500 ng	Adjust to 12.5 µL with buffer	1.50 µL	11.50 µL
Total volume: 12.5 µL		Total volume: 12.5 µL	
25 µL mRNA transfection complex			

6-well format

Tube 1		Tube 2	
mRNA	**StemMACS Transfection Buffer**	**StemMACS Transfection Reagent**	**StemMACS Transfection Buffer**
250 ng	Adjust to 50 µL with buffer	0.75 µL	49.25 µL
500 ng	Adjust to 50 µL with buffer	1.50 µL	48.50 µL
1000 ng	Adjust to 50 µL with buffer	3.00 µL	47.00 µL
Total volume: 50 µL		Total volume: 50 µL	
100 µL mRNA transfection complex			

5. Combine both dilutions by adding the diluted StemMACS Transfection Reagent to the diluted mRNA.
6. Mix by pipetting up and down. Do not vortex.
7. Incubate at room temperature for 20 minutes.
8. Add the mRNA transfection complex dropwise to the pre-plated cells (i.e. 25 µL of transfection complex per 24-well format, 100 µL per 6-well format). Keep the pipet tip above the surface of the media.
9. Gently rock the plates to ensure even distribution of the mRNA transfection complex.
10. Incubate cells under standard cell culture conditions.
11. After 4 hours, replace the cell culture media with fresh, pre-warmed medium.

    ▲ **Note:** This step is highly recommended, when working with sensitive cell lines or performing repeated daily transfections to minimize potential toxicity. The media replacement may be omitted when working with robust cell lines or single transfections.

12. Expression of the transfected factor will be detectable 6–24 hours after transfection depending on mRNA and protein stability.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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