Miltenyi Biotec

StemMACS™ Cre Recombinase mRNA

Order no. 130-101-113

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1. Description

Components	**20 µg StemMACS™ Cre Recombinase mRNA** encoding codon-optimized Cre recombinase. **1 mL Double-distilled Water,** RNase-free
Specifications	*In vitro* transcribed polyadenylated and capped mRNA that has been modified with pseudouridine and 5-methyl-cytidine to reduce the innate antiviral response to single-stranded RNA.
Formulation	Lyophilized from a filtered (0.2 µm) solution.
Storage	Store the lyophilized product at –20 °C. The expiration date is indicated on the label. After reconstitution, the product can be stored at –70°C for up to 3 month.
Quality control	mRNA size has been verified on an Agilent Bioanalyzer System. Cre protein expression after transfection was confirmed by flow cytometric analysis of transfected cells.

1.1 Principle

The transient expression of key developmental regulators, recombinases or markers via synthetic mRNA transfection is a powerful tool for modulating cell fate. StemMACS mRNAs are highly pure, *in vitro*-transcribed mRNAs that have been carefully optimized and validated to ensure high level expression after transfection.

1.2 Background information

Cre recombinase is a tyrosine recombinase enzyme derived from the P1 bacteriophage that catalyzes site-specific recombination between two DNA recognition sites (loxP sites).

1.3 Applications

- Site-specific recombination between loxP sites

2. Protocol: Reconstitution of lyophilizate

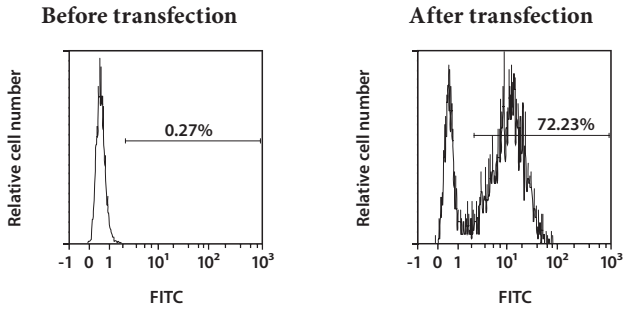
▲ RNA is susceptible to degradation by exogenous ribonucleases. Wear gloves, use RNase-free reagents, tubes, and pipette tips.

1. Dissolve StemMACS Cre Recombinase mRNA in 200 µL of Double-distilled Water. Vortex thoroughly. The final concentration will be 0.1 µg/µL.
2. Briefly centrifuge to collect the content at the bottom of the tube.
3. Prepare aliquots and store at –70 °C to –80 °C. Do not subject aliquots to more than two freeze-thaw cycles.

For satisfactory transfection results, use a protocol that is optimized for your specific cell type. StemMACS eGFP mRNA or StemMACS Nuclear eGFP mRNA allow easy evaluation of transfection efficiency and are recommended as positive controls.

3. Example of cre-mediated recombination in a mouse ES cell line

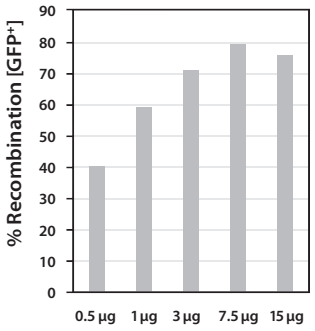
HM1 mouse embryonic stem cells carrying a GFP reporter gene that is transcribed upon cre-mediated recombination were electroporated with 3 µg StemMACS Cre Recombinase mRNA and analyzed by flow cytometry 24 hours after transfection. 72% of all cells were GFP-positive indicating successful cre-mediated recombination. Transfection efficiency was >80% as determined by independent electroporation of StemMACS eGFP mRNA into control HM1 cells (not shown).



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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The optimal amount of StemMACS Cre Recombinase mRNA was determined by titration. A single electroporation of the HM1 reporter cell line with >3 µg StemMACS Cre Recombinase mRNA resulted in 70–80% recombination.



Recombination and transfection efficiency will depend on the cell line and transfection technology used. For best results, monitor transfection efficiency using StemMACS eGFP mRNA and determine the optimal amount of StemMACS Cre Recombinase mRNA by titration.

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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