Miltenyi Biotec

StemMACS™ Flp Recombinase mRNA

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1. Description

Components	**20 µg StemMACS™ Flp Recombinase mRNA** encoding a codon-optimized version of Flp recombinase (FlpO). **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 months.
Quality control	mRNA size has been verified on an Agilent Bioanalyzer System. Flp protein expression and function after transfection was confirmed by eGFP exision in a transgenic HM1 mouse embryonic cell line carrying a single copy of eGFP flanked by two flippase recognition target (FRT) sites.

1.1 Principle

The transient expression of key developmental regulators, recombinases or markers via 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

Flippase (Flp) is a site-specific recombinase derived from the yeast *Saccharomyces cerevisiae*. It mediates recombination between two identical flippase recognition target (FRT) sites.

1.3 Applications

- Site directed recombination between FRT-sites.

2. Protocol: Reconstitution of lyophilizate

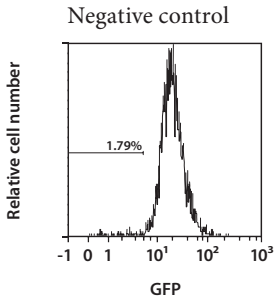
▲ RNA is susceptible to degradation by exogenous ribonucleases. Wear gloves, use RNase-free reagents, tubes, and pipette tips.

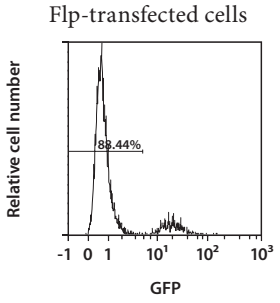
1. Dissolve StemMACS Flp Recombinase mRNA in 200 µL of Double-distilled Water. Vortex thoroughly. The final concentration will be 0.1 µg/µL.
2. Briefly centrifuge to collect the content at the bottom of the tube.
3. Prepare aliquots and store at –70 °C to –80 °C. Do not subject aliquots to more than 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 FlpO-mediated recombination in a mouse ES cell line

HM1 mouse embryonic stem cells carrying a GFP reporter gene flanked by two flippase recognition target (FRT) sites were electroporated with 15 µg StemMACS Flp Recombinase mRNA and analyzed by flow cytometry 96 hours after transfection. 88% of all cells were GFP-negative indicating successful Flp-mediated recombination. Transfection efficiency was >90% as determined by independent electroporation of mCherry mRNA into control HM1 cells (not shown).





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