Miltenyi Biotec

StemMACS™ iPSC mRNA Reprogramming Kit
human

Order no. 130-132-990

Contents

1. Description

This product is for research use only.

Components

1× StemMACS mRNA Reprogramming Box, human containing:

2×25 µg StemMACS mRNA Reprogramming Cocktail, human

2×1 mL Double-distilled water

1×130 µL StemMACS mRNA Transfection Reagent

1×25 mL StemMACS mRNA Transfection Buffer

1× StemMACS Repro-Brew XF, human containing:

1×500 mL StemMACS Repro-Brew Basal Medium XF, human

1×10 mL StemMACS Repro-Brew Supplement XF (50×), human

Specifications

StemMACS Repro-Brew XF, human:
pH: 7.2–7.6
Osmolality: 300–330 mOsmol/kg

Quality control

Functionality-tested for reprogramming of human fibroblasts into iPSCs.

Storage

Upon arrival, store lyophilized StemMACS mRNA Reprogramming Cocktail, human at –20 °C. After reconstitution, the cocktail can be stored at –70 °C to –80 °C for up to three months.
Store StemMACS Repro-Brew Basal Medium XF, human protected from light at 2–8 °C. Do not freeze.
Store StemMACS Repro-Brew Supplement XF (50×), human at –20 °C. Avoid repeated freeze-thaw cycles.
Store StemMACS mRNA Transfection Reagent and StemMACS mRNA Transfection Buffer at 2–8 °C. Do not freeze.
The expiration dates are indicated on the labels.

1.1 Background information

The StemMACS iPSC mRNA Reprogramming Kit, human has been designed for the rapid and efficient reprogramming of human fibroblasts into induced pluripotent stem cells (iPSCs). With the StemMACS iPSC mRNA Reprogramming Kit, human iPSCs can be generated within 14 days under feeder-free conditions without the need for a conditioned medium or the use of B18R protein. In contrast to traditional viral- or DNA-based reprogramming methods, mRNA reprogramming eliminates the risk for genomic integration and the safety concerns connected with using viral-based vectors. StemMACS mRNA Reprogramming Cocktail, human contains transcripts of the following genes: *POU5F1 (OCT3/4), SOX2, KLF4, MYC, NANOG, LIN28A*, and *SOCS1*.

1.2 Applications

- mRNA-based reprogramming of human fibroblasts into iPSCs

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2. Protocol

2.1 Preparation of complete medium

▲ Before StemMACS Repro-Brew XF, human can be used in cell culture, the two components need to be mixed according to the following protocol to obtain the complete medium.

1. Thaw StemMACS Repro-Brew Supplement XF (50×), human at room temperature prior to use. If a precipitate is visible at the bottom of the vial, resuspend thoroughly or swirl the vial in a 37 °C waterbath for 1–2 minutes until the supplement is clear.
2. Prepare appropriate aliquots and store them at –20 °C. Avoid repeated freeze-thaw cycles.
3. To obtain the complete medium, dilute StemMACS Repro-Brew Supplement XF (50×), human 1:50 with StemMACS Repro-Brew Basal Medium XF, human, e.g., add 2 mL StemMACS Repro-Brew Supplement XF (50×), human to 98 mL StemMACS Repro-Brew Basal Medium XF, human. The complete medium can be used for up to 14 days when stored at 2–8 °C.

2.2 Reconstitution of StemMACS mRNA Reprogramming Cocktail, human

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

1. Dissolve 25 µg StemMACS mRNA Reprogramming Cocktail, human in 250 µL of double-distilled water. Vortex thoroughly. The final concentration is 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. Avoid repeated freeze-thaw cycles. Thawed aliquots can be stored at 2–8 °C for up to five days.

2.3 Reprogramming of fibroblasts

▲ The detailed protocol is available at **www.miltenyibiotec.com/reprogramming-fibroblasts**.

The reprogramming protocol includes plating of fibroblasts in complete medium and allowing them to adapt. mRNA transfections are performed twice a day for five consecutive days. After several days of medium changes iPSC colonies can be isolated for further expansion.

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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