StemMACS™ DiffBase XF
human

Order no. 130-126-015

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1. Description

This product is for research use only.

Components	**500 mL StemMACS iPS-Brew Basal Medium XF, human** **10 mL StemMACS DiffBase Supplement XF (50×), human**
Specifications	Osmolality: 300–340 mOsmol/kg pH: 7.2–7.6
Storage	Store StemMACS iPS-Brew Basal Medium XF, human protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. Upon arrival store StemMACS DiffBase Supplement XF (50×), human at –20 °C. Aliquots of the supplemented complete medium can be stored at –20 °C for up to 2 months. Avoid repeated freeze-thaw cycles. Once thawed, aliquots should be kept at 2–8 °C and used within 2 weeks. The expiration date is indicated on the vial label.

1.1 Background information

StemMACS DiffBase XF, human is xeno-free and is an optimized and standardized base medium for the differentiation of human pluripotent stem cells (hPSCs). StemMACS DiffBase XF has been developed to be used directly on hPSCs cultivated in StemMACS iPS-Brew XF and can be used for spontaneous differentiation assays, such as the embryoid bodies (EBs) assay, or as a base medium for virtually every type of 2D or 3D directed differentiation protocol. In fact, StemMACS DiffBase XF supports cellular survival and successful differentiation of hPSCs both in differentiation protocols based on monolayer adherent culture and protocols that need a tridimensional EB-intermediate. Cellular fate–specific patterning factors, such as cytokines and small molecules, are available separately.

1.2 Applications

- Spontaneous differentiation of hPSCs as tridimensional aggregates: embryoid body assay.
- Directed differentiation of hPSCs via embryoid body formation (3D differentiation).
- Directed differentiation of hPSCs as adherent cultures (2D differentiation).

1.3 Reagent and instrument requirements

- Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}
- Trypsin/EDTA (alternatively, Accutase® or TrypLE™)
- Soybean Trypsin Inhibitor (0.5 mg/mL)
- Rho kinase inhibitors: StemMACS Y27632 (# 130-103-922) to generate embryoid bodies or StemMACS Thiazovivin (# 130-104-461) to improve cell attachment and survival in adherent directed differentiation
- Culture ware: Ultra-low attachment plates (for embryoid body formation), e.g. Corning (#3262), tissue culture–treated plates (for 2D differentiation assays)

▲ **Note:** Additional reagents and consumables might be needed according to the cell type–specific differentiation protocol used.

2. Protocol

2.1 Preparation of complete medium

Before StemMACS DiffBase XF can be used in cell culture, the two medium components need to be mixed according to the following protocol to obtain the complete medium.

1. Thaw StemMACS DiffBase Supplement XF (50×) at room temperature prior to use or overnight at 2–8 °C. Thawed material must be used immediately.
2. To obtain the complete medium add 10 mL StemMACS DiffBase Supplement XF (50×) to 500 mL StemMACS iPS-Brew Basal Medium XF. Mix well. The medium is ready-to-use now. Use the complete medium within 2 weeks when stored at 2–8 °C.
3. For longer storage, prepare 50 mL aliquots and store at –20 °C for up to 2 months. Thaw aliquots of complete medium overnight at 2–8 °C. Once thawed, keep aliquots at 2–8 °C and use within 2 weeks.

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

140-005-812.03

2.2 Harvesting of human pluripotent stem cells

▲ Volumes given below are for PSCs cultured in 6-well plates. If using different culture ware, scale accordingly.

1. Aspirate cell medium and wash each well with 3 mL of DPBS.
2. Add 0.7 mL of 0.05% Trypsin/EDTA per well. Gently rock the plate to ensure even distribution of the enzyme solution.
3. Incubate for 5 minutes at 37 °C.
4. Stop enzymatic reaction by adding 2 mL of Soybean Trypsin Inhibitor (0.5 mg/mL) per well.
5. Using a 5 mL serological pipette, dissociate to a single-cell suspension by carefully pipetting up and down.
6. Determine cell number.

2.3 Spontaneous differentiation (embryoid body formation assay)

1. Follow the protocol 2.2 Harvesting of human pluripotent stem cells.
2. Transfer the desired cell number into a 15 mL conical tube.

   ▲ **Note:** For embryoid body formation it is recommended to use 5×10^6 cells per 10 cm ultra-low attachment dish. Nevertheless, a cell line–dependent titration might be necessary.
3. Centrifuge for 5 minutes at 200×g.
4. Aspirate supernatant.
5. Resuspend the cell pellet in 12 mL complete StemMACS DiffBase XF medium supplemented with 10 µM StemMACS Y27632 per 10 cm ultra-low attachment plate.
6. Place the plate into the incubator and distribute the cells equally within the plate by gently swinging the plate.
7. Perform a medium change with fresh complete StemMACS DiffBase XF medium every second day.
8. Transfer embyoid bodies (EBs) into a 15 mL conical tube.
9. Spin sample for 15 seconds at 200×g to sediment EBs.
10. Carefully aspirate supernatant.
11. Resuspend in 12 mL of fresh complete StemMACS DiffBase XF medium.
12. When placing the dish back into the incubator, make sure to always distribute the cells equally within the dish by gently swinging the plate.

    ▲ **Note:** From day 8 embryoid bodies could be settled down by gravity by leaving them undisturbed in a 15 mL conical tube for 5 minutes.

2.4 Directed differentiation

StemMACS DiffBase XF has been developed as a base differentiation medium for PSCs cultured in StemMACS iPS-Brew XF. Therefore, cells do not need to adapt to the new medium and differentiation can start immediately.
Directed differentiation can be carried out by timely addition of cytokines and small molecules according to the differentiation protocol of choice either via monolayer adherent culture or tridimensional embryoid body–intermediate.
Harvest hPSCs as described in chapter 2.2. When starting the differentiation, it is recommended to supplement the medium with StemMACS Y27632 to generate embryoid bodies or StemMACS Thiazovivin to improve cell attachment and survival in adherent differentiation. Transfer the cells either into ultra-low attachment plates (for embryoid body formation) or tissue culture–treated plates (for adherent differentiation assays).

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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