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1. Description

This product is for research use only.

Components	8 mL StemMACS PSC-Support XF (25×), human			
Specifications	рН: 7.2–7.6			
Quality control	Low endotoxin level by Limulus Amoebocyte			
	Lysate (LAL) assay. Tested negative for			

mycoplasma.StorageUpon arrival, store StemMACS PSC-Support
XF, human at -20 °C. Aliquots can be stored
at -20 °C for up to six months. Avoid repeated
freeze-thaw cycles. Once thawed, aliquots
should be kept at +2 to +8 °C and be used within
two weeks. The expiration date is indicated on
the vial label.

1.1 Background information

StemMACS PSC-Support XF, human is a carefully optimized supplement that improves human pluripotent stem cell (hPSC) culture without affecting their pluripotent phenotype.

StemMACS PSC-Support XF has a xeno-free formulation and can be combined with StemMACS PSC-Brew XF, human. Their synergistic action allows an improved cell survival and a reliable restart of proliferation even at low cellular plating densities. Therefore, StemMACS PSC-Support XF combined with StemMACS PSC-Brew XF can be used in challenging applications that would normally stress the culture, such as gene editing approaches, flow sorting, or after reprogramming.

1.2 Applications

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• Improvement of hPSC survival and proliferation in challenging culturing conditions.

StemMACS[™] PSC-Support XF

human

Order no. 130-127-287

1.3 Reagent requirements

- StemMACS PSC-Brew XF, human (# 130-127-865) containing StemMACS PSC-Brew Basal Medium XF and StemMACS PSC-Brew 50× Supplement XF
- Cell attachment substrate. Recommended substrates are, e.g., MACSmatrix Laminin 511, Corning[®] Matrigel[®], hESC-Qualified Matrix, or Laminin 521.

2. Protocol

▲ Further protocols are available at https://www.miltenyibiotec.com/ applications/pluripotent-stem-cells.html.

2.1 Preparation of supplemented PSC medium

▲ Before StemMACS PSC-Support XF can be used in cell culture, it needs to be combined with a PSC culture medium. When used in combination with StemMACS PSC-Brew XF, human the following protocol is recommended.

StemMACS PSC-Support XF comes as a 25× stock solution. Dilute it 25-fold into the maintenance medium as follows:

- 1. Thaw StemMACS PSC-Support XF at +2 to +8 °C.
- 2. Mix thoroughly until no residuals are visible at the bottom to ensure complete thawing.
- 2. Add 8 mL StemMACS PSC-Support XF, to 200 mL StemMACS PSC-Brew XF to obtain the supplemented PSC medium.
- 3. Mix well. Supplemented PSC medium can be used for up to one week when stored at +2 to +8 °C. Do not freeze.

2.2 Coating of culture ware

Prepare 96-well culturing plates according to the manufacturer's protocol when Corning[®] Matrigel[®] hESC-Qualified Matrix is used. When using full-length Laminins or fragments use a concentration of 1.5 μ g/cm² and add a total volume of 50 μ L per well.

2.3 Plating protocol

Preparation of culture ware

▲ Equilibrate coated plates to room temperature before proceeding.

- 1. Aspirate the coating solution.
- 2. Add 50 µL/well of supplemented PSC medium.
 - ▲ Note: Make sure that the entire surface is covered with the medium.
- 3. Incubate at +37 °C for 1 hour prior to seeding cells.

Preparation of cell suspension

- 1. Harvest the cells and obtain a single cell suspension.
- 2. Determine cell number.
- 3. Adjust the cell concentration recommended for the respective assay by diluting the cell suspension in supplemented PSC medium. The final volume needs to be 50 μ L/well independently of the total cell number chosen.
- 4. Add 50 μ L/well of the diluted cell suspension to the previously prepared and pre-warmed 96-well plates for a total volume of 100 μ L/well.
- 5. Rock the plates gently to distribute the cells.
- 6. Incubate the plate at +37 °C. Do not disturb.

Media change strategy

- 1. After 48 hours, perform a full medium change with supplemented PSC medium (100 μ L/well).
- 2. At day 3, add 50 µL/well of supplemented PSC medium.
- 3. From day 4 on, perform medium changes every other day using just StemMACS PSC-Brew XF.

▲ Note: From day 4 on, it is not recommended to use StemMACS Support XF anymore.

Day	Action	Medium	Volume	
-1	Coat plates			
	Prepare media			
0	Plate cells at the desired density	StemMACS PSC-Brew XF supplemented with StemMACS PSC-Support XF	100 μL/well	
1	Leave plates undisturbed			
2	Full medium change	StemMACS PSC-Brew XF supplemented with StemMACS PSC-Support XF	100 μL/ well	
3	Medium addition	StemMACS PSC-Brew XF supplemented with StemMACS PSC-Support XF	50 μL/ well	
4	Full medium change	StemMACS PSC- Brew XF	100 µL/ well	
Every other day	Full medium change	StemMACS PSC- Brew XF	100 μL/ well	

Table 1: Protocol timeline and media change strategy for 96-well plates.

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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