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# 1. Description

#### This product is for research use only.

Components	100 mL StemMACS HSC Expansion Medium XF, human
	or
	500 mL StemMACS HSC Expansion Medium XF, human
Specifications	Osmolality: 320–350 mOsmol/kg pH: 7.0–7.5
Quality control	Functionality assay: Satisfactory <i>ex vivo</i> expansion of human CD34 <sup>+</sup> cells.
Storage	Store protected from light at -20 °C. The expiration date is indicated on the label. To avoid repeated freeze-thaw cycles, StemMACS HSC Expansion Medium XF, human should be aliquoted to the preferred sample volume, and stored at -20 °C until use.

#### 1.1 Background information

StemMACS HSC Expansion Medium XF, human is an optimized and standardized medium for the expansion of isolated CD34<sup>+</sup> hematopoietic stem and progenitor cells. The medium formulation is serum-free and xeno-free and is manufactured under strictly controlled conditions. StemMACS HSC Expansion Medium XF offers consistent lot-to-lot performance. Recombinant growth factors, required for the optimal growth and expansion of hematopoietic stem cells (HSCs), have not been added to the medium. They are available as separate product and have to be added prior to use. StemMACS HSC Expansion Cocktail (# 130-100-843) contains a combination of recombinant human cytokines designed to support the proliferation of human hematopoietic progenitor cells. It contains recombinant human stem cell factor (SCF), Flt3-ligand, and thrombopoietin (TPO). This combination of cytokines induces the proliferation of HSCs and immature progenitors.

#### 1.2 Applications

• Expansion of CD34<sup>+</sup> cells from cord blood, peripheral blood, or bone marrow.

# StemMACS<sup>™</sup> HSC Expansion Medium XF

# human

100 mL 500 mL 130-100-473 130-100-463

#### 1.3 Reagent requirements

- StemMACS HSC Expansion Cocktail (# 130-100-843)
- (Optional) CD34 MicroBead Kit, human (# 130-046-702) or CD34 MicroBead Kit UltraPure, human (# 130-100-453)

# 2. Protocol

#### 2.1 Preparation of cells

- 1. Isolate hematopoietic stem and progenitor cells using standard methods, e.g., the CD34 MicroBead Kit, human (# 130-046-702) or CD34 MicroBead Kit UltraPure, human (# 130-100-453).
- 2. After separation, determine CD34<sup>+</sup> cell purity and cell number by flow cytometry.

#### 2.2 Preparation of StemMACS HSC Expansion Medium XF

▲ To avoid repeated freeze-thaw cycles, StemMACS HSC Expansion Medium XF should be dispensed into appropriate aliquots.

- 1. Thaw medium bottle overnight at 4 °C.
- 2. Mix medium by gentle rotation of the bottle.
- 3. Aliquot into sterile tubes.
- 4. Freeze aliquots at -20 °C.
- 5. Prior to use thaw the medium at room temperature and add StemMACS HSC Expansion Cocktail at a 100× dilution.

#### 2.3 Expansion

1. For expansion seed the CD34<sup>+</sup> cells in StemMACS HSC Expansion Medium XF supplemented with StemMACS HSC Expansion Cocktail.

▲ Note: The optimal number of cells plated, culture period, and feeding intervalls will depend on experimental set-up and should be determined individually. For CD34<sup>+</sup> cells from cord blood, e.g., a cell density of 5000 cells/mL is recommended.

2. Incubate cells at 5 % CO<sub>2</sub> and 37 °C.

#### 2.4 Analysis

After expansion, cells should be counted and analyzed for the expression of hematopoietic markers, such as CD45, CD34, CD133, CD38, or CD45RA.

The functionality of the expanded cells can be analyzed, e.g., by CFU assays, LTC-IC assays, or transplantation into immunodeficient mice.



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