Miltenyi Biotec

StemMACS™ HSC-CFU Media
human

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1. Description

This product is for research use only.

Components

Product	Filling volume	Order no.
StemMACS HSC-CFU complete with Epo, human	100 mL	130-091-280
StemMACS HSC-CFU lite with Epo, human	100 mL	130-091-281

Quality control CFU assay

Storage Store protected from light at –20 °C. The expiration date is indicated on the bottle label.

1.1 Background information

The hematopoietic system is constantly self-renewing and comprised of cells at various stages of maturation. These include rare primitive stem cells with multi-lineage differentiation capacity and high self-renewal as well as progenitor cells with restricted differentiation and self-renewal potential.

Initial culture systems used marrow stromal cell layers consisting of reticular cells, fibroblasts, macrophages, and adipocytes to support *in vitro* growth of hematopoietic stem cells (HSCs).[1,2] Stromal cells were thought to exert their effects through the production of extracellular matrix and the secretion of cytokines.[3,4]

Today, semi-solid culture media have become the standard for enumeration and evaluation of stem and progenitor cells as colony-forming units (CFU). Based on methylcellulose in IMDM and supplemented with fetal bovine serum (FBS) and different growth factors, these media mimic the effect of stromal cells and provide optimal growth conditions.

StemMACS HSC-CFU Media have been developed to maximize growth and differentiation of progenitor cells and allow the clonal progeny of a single-cell to grow in a distinct cluster or colony. They are produced under tightly controlled manufacturing conditions using highly qualified raw materials to provide a consistent and optimally performing CFU assay medium.

1.2 Product formulation

	HSC-CFU complete with Epo	HSC-CFU lite with Epo
The formulation supports growth of:		
CFU-G, CFU-M, CFU-GM	yes	yes
BFU-E, CFU-E, CFU-GEMM	yes	yes
Components		
Methylcellulose in Iscove`s MDM	1%	1%
Fetal bovine serum (FBS)	30%	30%
Bovine serum albumin (BSA)	1%	1%
L-glutamine	2 mM	2 mM
2-mercaptoethanol	0.1 mM	0.1 mM
Stem cell factor (SCF)	50 ng/mL	50 ng/mL
GM-CSF	20 ng/mL	10 ng/mL
G-CSF	20 ng/mL	–
IL-3	20 ng/mL	10 ng/mL
IL-6	20 ng/mL	–
Erythropoietin (Epo)	3 U/mL	3 U/mL

1.3 Reagent and instrument requirements

- Dilution medium: Iscove's Modified Dulbecco's Medium (IMDM) with 2% FBS.
- Sterile 15 and 50 mL polypropylene tubes
- Sterile pipettes and 3–5 mL syringe
- Sterile 16-gauge blunt-end needle
- 35 mm cell culture dishes and 100 mm Petri dishes (Alternatively use 6-well plates with low-evaporation lid)

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

140-001-101.09

2. Protocol

2.1 Preparation of StemMACS HSC-CFU Media

To avoid repeated freeze-thaw cycles, StemMACS HSC-CFU Media should be dispensed into appropriate aliquots.

1. Thaw media overnight at 4 °C.
2. Shake the bottle vigorously and let it stand for 10–20 minutes to allow air bubbles to rise to the top.
3. Aliquot into sterile tubes (3 mL/tube) using a syringe attached to a 16-gauge blunt-end needle.
   ▲ **Note:** Do not use a standard pipette as the volume dispensed will not be accurate.
4. Freeze aliquots at –20 °C. Thaw at room temperature before use.
   ▲ **Note:** Methylcellulose media should not be thawn at 37 °C!

2.2 Preparation of cell samples

Hematopoietic colony-forming unit (CFU) assays can be performed using mononuclear cells from bone marrow, spleen, and peripheral blood. Likewise, enriched hematopoietic stem and progenitor cells, e.g. enriched lineage marker-negative (Lin^-), $CD133^+$, or $CD34^+$ cells, or ES and iPS cell–derived progenitors can be used.

For details about the preparation of mononuclear cells refer to the protocols section at www.miltenyibiotec.com/protocols.

For pre-enrichment of $CD133^+$, $CD34^+$, or Lin^- cells, refer to the data sheet of the respective separation product.

2.3 Set-up of CFU assay

1. Thaw aliquots of StemMACS HSC-CFU Media at room temperature.
2. Adjust cell samples to a concentration which is 10-fold higher than the recommended final plating concentration (refer to table 1). Use IMDM with 2% FBS for dilution.

Cell source	Recommended final plating concentration [cells per 35 mm dish]
$CD34^+$, $CD133^+$, or Lin^- cells	500 (150–1000)*
Peripheral blood mononuclear cells (PBMC)	2×10^5 (1×10^5–2×10^5)*
Cord blood mononuclear cells (CB-MNCs)	1×10^4 (5×10^3–2×10^4)*
Bone marrow mononuclear cells (BM-MNCs)	2×10^4 (1×10^4–5×10^4)*
Bone marrow (NH_4Cl-treated)	5×10^4 (2×10^4–1×10^5)*
Leukapheresis harvest (G-CSF or GM-CSF mobilized donor)	2×10^4 (5×10^3–5×10^4)*

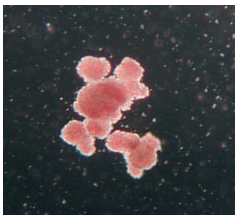
* Possible range of cell concentration to determine the optimal plating concentration.

Table 1: Recommended final plating concentrations per 35 mm dish.

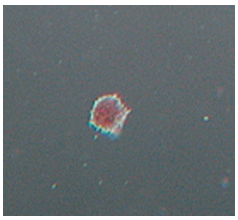
2. Add 0.3 mL of cells to a 3 mL aliquot of StemMACS HSC-CFU Media immediately prior to plating.
3. Vortex the tube vigorously until the cells are well suspended and let tubes sit for about 10 minutes to allow air bubbles to rise.
4. Using a sterile 3 mL syringe fitted with a 16-gauge blunt-end needle, aliquot 1.1 mL of the cell/methylcellulose suspension into each of two 35 mm petri dishes. Avoid air bubbles.
5. Rotate the dishes gently in order to spread the suspension evenly over the plate.
6. Place pairs of 35 mm dishes in a 100 mm dish. Add a third 35 mm dish without lid containing 3 mL sterile water to the 100 mm dish in order to maintain an adequately humified atmosphere during culturing.
   ▲ **Note:** It is also possible to use 6-well plates with low-evaporation lids. Fill two of the wells with sterile water.
7. Incubate the plate for 14–16 days in an humified incubator at 37 °C and 5% CO_2.
   ▲ **Note:** Place the plate in an area that will not be disturbed for the duration of incubation.

2.4 Reading the plate

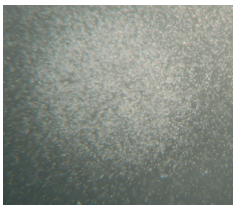
Hematopoietic colonies can be classified by color and morphology using a stereoscope or an inverted microscope.



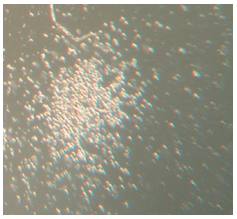
BFU-E (burst-forming unit – erythroid: >200 early erythrocyte progenitor cells are typically found in 3–8 densely packed clusters. The color of hemoglobin-containing cells ranges from dark red/orange to brownish.



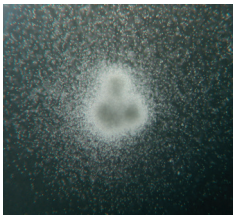
CFU-E (colony-forming unit – erythroid): 8–200 erythrocyte progenitor cells are typically found in 1–2 densely packed clusters. The color of hemoglobin-containing cells ranges from dark red/orange to brownish.



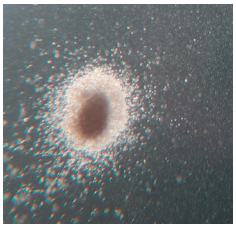
CFU-G (colony-forming unit – granulocyte): A flat colony consisting of 20–40 translucent small cells with a germinative center.



CFU-M (colony-forming unit – macrophage): A sparsely growing, flat colony consisting of >20 translucent large cells.



CFU-GM (colony-forming unit – granulocyte, macrophage): A flat colony consisting of 20–50 translucent small and large cells.



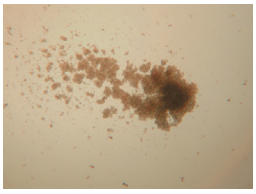
CFU-GEMM (colony-forming unit – granulocyte, erythrocyte, macrophage, megakaryocyte):
Has a compact area that is usually central to a peripheral flat lawn of translucent cells that may be either large or small ("fried egg" appearance). The color of hemoglobin-containing cells ranges from dark red/orange to brownish.

3. Appendix: Tips & hints

No erythroid colonies – Make sure to use a media formulation that contains Epo. Score colonies at day 12–16. If scored before, colonies may not be fully hemoglobinized yet and may appear granulopoietic.

No distinct clusters or colonies – Avoid agitation for the time of incubation. Place the plate in an area of the incubator that will not be disturbed for the duration of incubation.

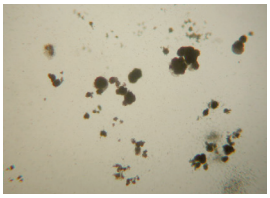
Example:



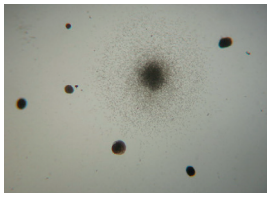
Colonies are too dense – Use a lower plating concentration when preparing the plate. Refer to table 1 for the recommended range of cell concentration.

Examples:

Colony density too high



Optimal colony density



4. References

1. Dexter, T. M. (1982) Stromal cell associated haemopoiesis. J. Cell Physiol. (suppl. 1): 87–94.
2. Dexter, T. M. *et al.* (1977) Conditions controlling the proliferation of haemopoietic stem cells *in vitro*. J. Cell Physiol. 91: 335–344.
3. Gordon, M. Y. *et al.* (1987) Compartmentalization of a haematopoietic growth factor (GM-CSF) by glycosaminoglycans in the bone marrow microenvironment. Nature 326: 403–405.
4. Dexter, T. M. (1987) Growth factors involved in haemopoiesis J. Cell Sci. 88: 1–6.

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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