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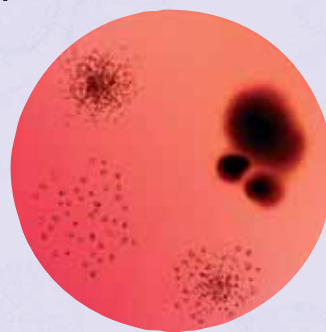
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Miltenyi Biotec

MACS® HSC-CFU Media human



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Unless otherwise specifically indicated,
Miltenyi Biotec products and services are for research use only
and not for diagnostic or therapeutic use.

Contents

Methylcellulose-based media for colony-forming-unit (CFU) assays of human hematopoietic stem and progenitor cells

HSC-CFU complete with Epo, human	100 mL	# 130-091-280
	24x3 mL	# 130-091-278
HSC-CFU complete w/o Epo, human	100 mL	# 130-091-277
	24x3 mL	# 130-091-276
HSC-CFU lite with Epo, human	100 mL	# 130-091-281
	24x3 mL	# 130-091-282
HSC-CFU basic, human	80 mL	# 130-091-275

All products should be stored at -20 °C.

The expiration dates are indicated on the bottle labels.

All products are for research use only!

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1. Products

MACS[®] HSC-CFU Media are developed for the enumeration of human hematopoietic stem cells (HSC) and progenitor cells characterized as colony-forming-units (CFU). Hematopoietic stem and progenitor cell CFU assays can be performed using mononuclear cells from bone marrow, cord blood, and peripheral blood. Also enriched hematopoietic stem and progenitor cells (CD34⁺ cells or CD133⁺ cells) as well as embryonic stem cells (ESCs) or induced pluripotent stem (iPS) cell-derived HSCs can be used. MACS HSC-CFU Media are standardized, semi-solid media that guarantee reliable and reproducible results in routine hematopoietic colony-forming assays.⁶

All media are rigorously tested for performance, stability, and lot-to-lot consistency.

HSC-CFU basic **80 mL** **# 130-091-275**

The HSC-CFU basic media do not contain cytokines and therefore allow the addition of growth factors or cytokines of choice. This provides the flexibility needed for non-standard applications.

Iscove's Modified Dulbecco's Medium (IMDM)

L-Glutamine (2 mM)
Methylcellulose (1%)
Fetal bovine serum (30%)
Bovine serum albumin (1%)
2-Mercaptoethanol (10⁻⁴ M)

▲ **Note:** Concentrations given above are not obtained until HSC-CFU basic medium is adjusted to a volume of 100 mL, by addition of growth factors, cytokines, and IMDM.

HSC-CFU complete with Epo **100 mL** **# 130-091-280**
24x3 mL **# 130-091-278**

The HSC-CFU complete with Epo medium supports the growth of granulocyte colonies (CFU-G), macrophage colonies (CFU-M), granulocyte/macrophage colonies (CFU-GM), erythroid colonies (CFU-E), burst-forming-units erythrocyte (BFU-E), and mixed colonies (CFU-GEMM).^{1,6,7,8}

Iscove's Modified Dulbecco's Medium (IMDM)

L-Glutamine (2 mM)
Methylcellulose (1%)
Fetal bovine serum (30%)
Bovine serum albumin (1%)
2-Mercaptoethanol (10⁻⁴ M)
Stem cell factor (50 ng/mL)
GM-CSF (20 ng/mL)
G-CSF (20 ng/mL)
IL-3 (20 ng/mL)
IL-6 (20 ng/mL)
Erythropoietin (3 U/mL)

HSC-CFU complete w/o Epo **100 mL** **# 130-091-277**
24x3 mL **# 130-091-276**

The HSC-CFU complete without Epo medium supports the growth of granulocyte colonies (CFU-G), macrophage colonies (CFU-M), and granulocyte/macrophage colonies (CFU-GM).¹

Iscove's Modified Dulbecco's Medium (IMDM)

L-Glutamine (2 mM)
Methylcellulose (1%)
Fetal bovine serum (30%)
Bovine serum albumin (1%)
2-Mercaptoethanol (10⁻⁴ M)
Stem cell factor (50 ng/mL)
GM-CSF (20 ng/mL)
G-CSF (20 ng/mL)
IL-3 (20 ng/mL)
IL-6 (20 ng/mL)

HSC-CFU lite with Epo **100 mL** **# 130-091-281**
24x3 mL **# 130-091-282**

The HSC-CFU lite with Epo medium supports the growth of granulocyte colonies (CFU-G), macrophage colonies (CFU-M), granulocyte/macrophage colonies (CFU-GM), erythroid colonies (BFU-E and CFU-E), and mixed colonies (CFU-GEMM).^{1,9}

Iscove's Modified Dulbecco's Medium (IMDM)

L-Glutamine (2 mM)
Methylcellulose (1%)
Fetal bovine serum (30%)
Bovine serum albumin (1%)
2-Mercaptoethanol (10⁻⁴ M)
Stem cell factor (50 ng/mL)
GM-CSF (10 ng/mL)
IL-3 (10 ng/mL)
Erythropoietin (3 U/mL)

Aliquoting of 80 or 100 mL bottles:

It is recommended that upon receiving the methylcellulose medium, it should be thawed overnight at 4 °C. For a complete resuspension, shake the bottle vigorously after thawing. Let the bottle stand for about 10–20 minutes to allow bubbles to rise to the top. Aliquot the media into sterile tubes (e.g. 3 mL/tube) using a syringe attached to a 16-gauge MACS Blunt-End Needle (# 130-091-558).

Freeze aliquots at –20 °C until use!**Avoid repeated freeze-thaw-cycles!**

Aliquots of HSC-CFU Media should be thawed at room temperature!

All procedures should be performed under sterile conditions!

2. Background information

The hematopoietic system is constantly self-renewing and thus comprises cells at various stages of maturation. It includes rare primitive stem cells, with multilineage differentiation capacity and high self-renewal as well as progenitor cells with restricted differentiation capacity and lack of self-renewal. Traditionally, marrow stromal cell layers consisting of reticular cells, fibroblasts, macrophages, and adipocytes have been utilized to support the *in vitro* growth of hematopoietic stem cells.^{2,3} Stromal cells have been thought to exert their effects through the production of the extracellular matrix and secretion of humoral factors *in vivo*.^{4,5} Since this system is time consuming and cumbersome, an alternative assay has been developed in which the *in vitro* differentiation of stem and progenitor cells is performed in semi-solid media that mimics the extracellular matrix.

These standardized media are based on methylcellulose in IMDM, supplemented with FCS and different growth factors. Methylcellulose-based culture media have become the standard media for enumeration and evaluation of stem and progenitor cells as colony-forming-units (CFU).⁶

The semi-solid MACS 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. MACS HSC-CFU Media are produced under tightly controlled manufacturing conditions using highly qualified raw materials to provide a consistent and optimally performing CFU assay medium.⁶

3. Reagent and instrument requirements

- Dilution medium: Iscove's Modified Dulbecco's Medium (IMDM) + 2% FBS.
- Sterile 15 and 50 mL polypropylene tubes
- Sterile pipettes and 3–5 mL syringe
- Sterile 16-gauge MACS Blunt-End Needle (# 130-091-558)
- Single 35 mm dishes
- Single 100 mm dishes
- (Optional) 6-well plates.

4. Cell samples

Hematopoietic colony-forming assays can be performed using mononuclear cells from cord blood, bone marrow, and peripheral blood or using enriched hematopoietic stem and progenitor cells (e.g. CD34⁺ cells or CD133⁺ cells) as well as ESC or iPS cell-derived HSCs.

For the preparation of cells from different cell sources, see the protocols section at www.miltenyi-biotec.com/protocols.

5. General protocol

5.1 Preparing the plate

- The cell samples should be adjusted to a concentration which is 10-fold higher than the recommended final plating concentration by using IMDM containing 2% FBS (see table 1).

Cell source	Recommended final plating concentration [cells/1.1 mL]
CD34 ⁺ or CD133 ⁺ cells	0.5–1×10 ³
PBMCs	2×10 ⁵
Cord blood mononuclear cells (CB-MNCs)	5×10 ³ –2×10 ⁴
Bone marrow mononuclear cells (BM-MNCs)	1×10 ⁴
Bone marrow (NH ₄ Cl-treated)	1×10 ⁵
Leukapheresis harvest (from a G-CSF or GM-CSF mobilized donor)	5×10 ³ –2×10 ⁴

Table 1: Recommended final plating concentration.

- Add 0.3 mL of cells to a 3 mL aliquot of HSC-CFU Medium in a sterile tube immediately prior to plating.
- Vortex the tube vigorously until the cells are well suspended and let tubes sit for about 10 minutes to allow air bubbles to rise.

- Using a sterile 3 mL syringe fitted with a 16-gauge MACS Blunt-End Needle (# 130-091-558), aliquot 1.1 mL of the cell/methylcellulose suspension into each of two 35 mm petri dishes. Avoid air bubbles.
- Rotate the dishes gently in order to spread the suspension evenly over the plate.
- Place pairs of 35 mm dishes in a 100 mm dish. Add a third 35 mm dish (without its lid) containing 3 mL sterile water to the 100 mm dish in order to maintain an adequately humidified atmosphere during culturing.

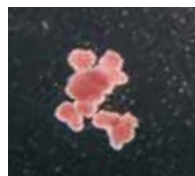
▲ **Note:** It is also possible to use 6-well plates. In that case use low-evaporation lids and fill two of the 6-well plates with water to avoid drying.

- Incubate the plate for 14–16 days in an humidified incubator at 37 °C and 5% CO₂.

▲ **Note:** Place the plate in area that will not be disturbed for the duration of incubation.

5.2 Reading the plate

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



BFU-E (burst-forming-unit erythrocyte):
>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 erythrocyte):
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.

6. References

- Williams, D.A. (2000) Hematology: Basic Principles and Practice, Hoffmann *et al.* (eds.) Churchill Livingstone, 126–138.
- Dexter, T.M. (1982) Stromal cell associated haemopoiesis. *J. Cell Physiol.* (suppl. 1): 87–94.
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- Watts, M. *et al.* (2008) *Cytotherapy* 10 (suppl. 1): Poster no. 150.
- Biedermann, B. *et al.* (2007) Analysis of the CD33-related siglec family reveals that Siglec-9 is an endocytic receptor expressed on subsets of acute myeloid leukemia cells and absent from normal hematopoietic progenitors. *Leuk. Res.* 31: 211–220.
- Del Fante, C. *et al.* (2005) Immunomagnetic cell selection performed for HLA haploidentical transplants with the CliniMACS device: effect of additional platelet removal on CD34+ cell recovery. *Stem Cells Dev.* 14: 734–739.
- Chang, K.-H. *et al.* (2006) Definitive-like erythroid cells derived from human embryonic stem cells coexpress high levels of embryonic and fetal globins with little or no adult globin. *Blood* 108: 1515–1523.

7. Appendix: Tips & hints

No erythroid colonies are found on the plate — use medium with Epo (erythropoietin). Another possible reason could be that the plate was scored too soon and erythroid colonies may not yet be hemoglobinized and appear as granulopoietic. The plate should not be evaluated until day 12–16 of cultivation.

Colonies do not stay in a distinct cluster or colony — place the plate in an area of the incubator that will not be disturbed for the duration of incubation.



Colonies on the plate are too dense — use lower cell concentration, when preparing the plate (see page 12, table 1).

Examples:

Colony density too high



Optimal colony density



8. Related products for human cells

CD133 MicroBead Kit (2×10 ⁹ total cells)	# 130-050-801
CD34 MicroBead Kit (2×10 ⁹ total cells)	# 130-046-702
CD34 MicroBead Kit (10 ¹⁰ total cells)	# 130-046-703
CD34 MultiSort Kit (2×10 ⁹ total cells)	# 130-056-701
CD117 MicroBead Kit (2×10 ⁹ total cells)	# 130-091-332
CD34-FITC	# 130-081-001
CD34-PE	# 130-081-002
CD34-APC	# 130-090-954
CD133/1 (AC133) pure	# 130-090-422
CD133/1 (AC133)-Biotin	# 130-090-664
CD133/1 (AC133)-PE	# 130-080-801
CD133/1 (AC133)-APC	# 130-090-826
CD133/2 (293C3) pure	# 130-090-851
CD133/2 (293C3)-Biotin	# 130-090-852
CD133/2 (293C3)-PE	# 130-090-853
CD133/2 (293C3)-APC	# 130-090-854

All protocols and data sheets are available at www.miltenyibiotec.com.

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