Cryopreserved Human Macrophages

PromoCell®

Instruction manual

Product	Size	Catalog number
Human M1 Macrophages (GM-CSF), monocyte-derived, single donor (hMDM-GMCSF(-))	1.5 million cryopreserved cells	C-12914
Human M2 Macrophages (M-CSF), monocyte-derived, single donor (hMDM-MCSF(-))	1.5 million cryopreserved cells	C-12915
Human M1 Macrophages (GM-CSF), monocyte-derived, single donor (hMDM-GMCSF(-))	5 million cryopreserved cells	C-12916
Human M2 Macrophages (M-CSF), monocyte-derived, single donor (hMDM-MCSF(-))	5 million cryopreserved cells	C-12917

Product description

Primary human macrophages (Mϕ) are hard to isolate from tissue in sufficient amounts with a homogeneous phenotype, do not proliferate in culture, and are difficult to handle *in vitro*. Our cryopreserved human monocyte-derived macrophages (hMDM) provide a convenient and easy-to-handle alternative. The thawed cells plate into all tissue culture vessel formats and can be maintained as adherent, biologically functional cultures for several weeks. Optionally, user-customizable activation of the cells can be performed (please refer to the culture protocol and table 1).
Cryopreserved human macrophages are produced from human monocytes in our well-proven M1/M2-Macrophage Generation Media XF and are available as non-activated, fully qualified M1- (GM-CSF) or M2- (M-CSF) polarized cells. After 9–10 days of differentiation, the M1/M2 macrophages are cryopreserved using our proprietary, defined, animal-component free, and protein-free cryopreservation medium, Cryo-SFM. Each cryovial contains more than 1.5 million or 5 million viable cells after thawing, respectively.

Quality control

We perform rigid quality control tests for each lot of cryopreserved human M1/M2 macrophages. The cells are tested for cell morphology, adherence rate, and viability. Furthermore, they are characterized by flow cytometric analysis of relevant markers namely CD80/CD68 for M1 macrophages (GM-CSF) and CD163/ CD68 for M2 macrophages (M-CSF). In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1 and HTLV-2, and microbial contaminants (fungi, bacteria, and mycoplasma). A detailed certificate of analysis (CoA) for each lot can be downloaded at:
www.promocell.com/coa

Intended use

Our cryopreserved human macrophages are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

Warning

Although tested negative for HIV-1, HIV-2, HBV, HCV, HTLV-1 and HTLV-2, the cells – like all products of human origin – should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

Follow appropriate safety precautions!

After delivery, cryopreserved cells should be stored in liquid nitrogen or seeded directly (see page 2).

Instructions for Cryopreserved Human Macrophages

Materials

- Cryopreserved hMDM-GMCSF(-) (C-12914, C-12916) or hMDM-MCSF(-) (C-12915, C-12917)
- M1- or M2-Macrophage Generation Medium XF (C-28055 or C-28056)
- Human fibronectin or vitronectin solution
- PBS without Ca^{2+}/Mg^{2+} (C-40232)
- 70% Ethanol
- Optional: human serum AB for long-term cultures of hMDM-MCSF (see II.D.3)

Use aseptic techniques and a laminar flow bench.

I. Culture protocol

1

Coat the culture vessel with human fibronectin or vitronectin

The culture vessel must be precoated either with 1 µg/cm² human fibronectin or 0.5 µg/cm² human vitronectin according to the instruction manual of the manufacturer. Use approx. 100 µl either of the diluted fibronectin solution (10 µg/ml) or 100 µl of the diluted vitronectin solution (5 µg/ml) per cm² of culture surface.

Note: Commercially available fibronectin-coated plasticware can also be used. Both fibronectin and vitronectin support the effective adhesion of macrophages to the growth surface. However, for a more enhanced and robust adhesion we rather recommend using fibronectin.

2

Prepare the complete medium in the coated culture vessel

Prepare the PromoCell M1/M2-Macrophage Generation Medium XF according to the instruction manual. Add an appropriate amount of medium (300–400 µl/cm², e.g. 3 ml per 6-well or 8 ml per T-25 flask) to the fibronectin- or vitronectin-coated vessel and pre-equilibrate for at least 30 minutes at 37°C and 5% CO_2 before seeding the cells.

Note: Use freshly prepared medium for best results. Macrophage Generation Medium XF with added cytokines used for plating of cryopreserved macrophages must not be older than one week at the time of plating. Discard complete medium older than two weeks.

3

Thaw the cryopreserved macrophages

Place 14 ml of fresh M1/M2-Macrophage Generation Medium XF (tempered 2–8°C) in a 15 ml conical tube and keep it under the laminar flow bench. This will be used as the thawing medium. Do not prewarm. Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation.

Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.

Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap.

Note: Work swiftly once the cells are thawed.

4

Equilibrate and count the thawed macrophages

Open the vial and transfer the cells to the 15 ml tube containing the thawing medium using a 2 ml serological pipet. Avoid introducing air bubbles during pipetting. Mix by a single gentle inversion and let the cells recover for 20 minutes at room temperature under the laminar flow bench.

After the recovery period determine the viable cell count using your standard method. Then, centrifuge the cells for 15 minutes at 350 x g at room temperature. Aspirate the super natant except for 50–100 µl. Loosen the cell pellet by grasping the top of the tube and firmly flicking its bottom 2–3 times. Then, resuspend the cells at 1 million cells/ml in fresh ambient tempered M1/M2-Macrophage Generation Medium XF using a serological pipet.

Note: Adhere to the stated centrifugation speed and time.

5

Plate the thawed macrophages

Immediately after thawing, plate the cell suspension at 100,000-200,000 cells/cm² in the fibronectin- or vitronectin-coated culture vessels containing the pre-equilibrated M1/M2-Macrophage Generation Medium XF. Leave the cells untouched in the incubator to allow for attachment of the macrophages to the culture surface for at least 4 hours (4–24 hours).

Note: A 70–90% confluent cell monolayer of M1 macrophages (GM-CSF) is routinely obtained with a seeding density of 100,000 cells/cm², while the smaller sized M2 macrophages (M-CSF) require approx. 200,000 cells/cm².

6

Replace the medium

Change the medium 4–24 hours after plating using approx. 300–400 µl fresh medium per cm² of culture surface. Discard residual non-adherent cells.

Allow the macrophages to stay in culture for at least 24 hours after seeding before starting your experiments. Optional activation of the polarized macrophages may be per formed 24 hours after thawing (please refer to table one for example concentrations).

In the following time, change the culture medium every two to three days. The macrophages can be maintained in culture for several weeks.

Note: As compared to GM-CSF, M-CSF acts as a weaker long-term macrophage survival factor. Accordingly, the integrity of M2 macrophage cultures to be maintained for over one week may optionally be improved by addition of human serum AB from day 5–7 onwards without affecting the polarization status of the cells. Simply add 2% (v/v) of human serum AB to fresh complete M2-Macrophage Generation Medium XF and filter aseptically through a 0.22 micron filter in order to remove the serum-associated lipid precipitate.

	Activation state	Former designation	Differentiation factor (day 0+)*	Activator (day 7+)	Activation process reference
M1	M(IFN-γ)	M1	GM-CSF (or M-CSF)	IFN-γ (50 ng/ml)	[1]
	M(LPS+IFN-γ)	M1	GM-CSF (or M-CSF)	IFN-γ (50 ng/ml) + LPS (10 ng/ml)	[1]
	M(LPS)	M1	GM-CSF (or M-CSF)	LPS (100 ng/ml)	[1]
	M(-)	M1, non-activated	GM-CSF	-	[2]
	M(-)	M0 / Mϕ	2% human AB serum	-	[1, 3]
	M(-)	M2, non-activated	M-CSF	-	[2]
	M(GC)	M2c	M-CSF	DEX (100 nM)	[2]
	M(TGFb)	M2c	M-CSF	TGF-β1 (20 ng/ml)	[2]
	M(IL-10)	M2c	M-CSF	IL-10 (10 ng/ml)	[4]
	M(IC+LPS)	M2b	M-CSF	IgG (immobilized) + LPS (100 ng/ml)	[5]
	M(IL-4)	M2a	M-CSF	IL-4 (20 ng/ml)	[4, 5]
M2	TAM	M2-like	tumor microenvironment	tumor microenvironment	[6]

Table 1: Human macrophage activation reference table according to the common framework consensus nomenclature [7]. *The published differentiation factor/activator combinations are listed to serve as a basic guidance. Specific effects of activation on macrophages*

DEX = dexamethasone, IC = immune complexes, IFN = interferon, IgG = immunoglobulin G, GC = glucocorticoids, (G)M-CSF = (granulocyte/)macrophage colony stimulating factor, IL = interleukin, LPS = lipopolysaccharide, TAM = tumor associated macrophages, TGF = transforming growth factor.

Specifications

Product	Recommended maintenance medium*	Plating density**	Marker
Human M1 Macrophages (GM-CSF), monocyte-derived, single donor (hMDM-GMCSF(-))	C-28055	100,000 cells per cm^2	$CD80^+$, $CD68^+$
Human M2 Macrophages (M-CSF), monocyte-derived, single donor (hMDM-MCSF(-))	C-28056	200,000 cells per cm^2	$CD163^+$, $CD68^+$

** The plated cells do not proliferate. ** Recommeded plating density for obtaining an adherent cell monolayer with 70–90% confluency.*

Related products

Product	Size	Catalog number
M1-Macrophage Generation Medium XF	250 ml	C-28055
M2-Macrophage Generation Medium XF	250 ml	C-28056
Macrophage Base Medium XF	250 ml	C-28057
Cryo-SFM	125 ml	C-29912
Dulbecco's PBS without Ca^{++}/Mg^{++}	500 ml	C-40232

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