Monocytes

PromoCell

Instruction Manual

Product	Size	Catalog Number
Human CD14$^+$ Monocytes from Peripheral Blood (hMoCD14$^+$-PB), single donor	10 x 10^6 cryopreserved cells	C-12909

Product Description

Monocytes (Mo) are immature phagocytic cells circulating in the blood. Acting as antigen-presenting immune cells, they can phagocytize and degrade microbes and particulate matter. Monocytes can differentiate into several different cell types. The most common applications for Monocytes are the *in vitro* differentiation into monocyte-derived dendritic cells (moDC), macrophages, and osteoclasts as well as their use as phagocytic immune cells in immunology and infection biology.

CD14 belongs to the family of LPS receptor antigens and is strongly expressed on the majority of Monocytes. Thus, binding of immunomagnetic particles to this antigen is employed for purification of PromoCell CD14$^+$-Monocytes (hMoCD14$^+$-PB) from Mononuclear Cells (MNC).

PromoCell Human CD14$^+$ Monocytes are isolated from fresh peripheral blood. After isolation of the ultra-pure mononuclear cell fraction by proprietary methods, they are isolated by positive selection.
Immediately after isolation, the freshly prepared human monocytes are cryopreserved using PromoCell´s proprietary serum-free, freezing medium Cryo-SFM. Each cryo vial contains more than 10 million viable cells after thawing.

Quality Control

Rigid quality control tests are performed for each lot of PromoCell Monocytes. They are routinely characterized by flow cytometry analyzing the viability, cell size and purity.
In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1, 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

PromoCell Monocytes 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, start immediately with the protocol for cryopreserved cells (see page 2).

Start immediately after delivery.
Use aseptic techniques and a laminar flow bench.

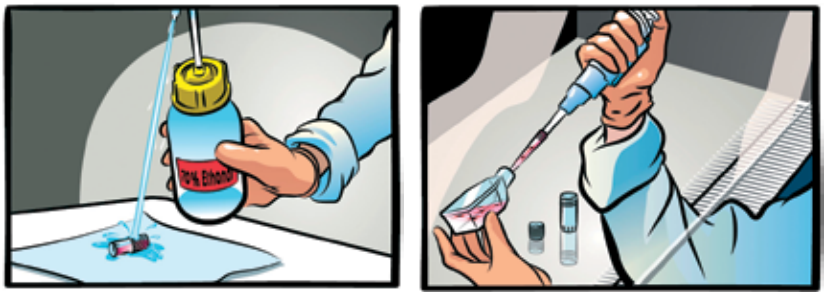
Protocol for Cryopreserved Cells

Straight after arrival, store the cryopreserved cells in liquid nitrogen or seed them immediately.
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

1. **Prepare the medium**
   Refer to the recommended seeding density (see page 4) and the cell number per vial given in the lot-specific CoA of the cells. Transfer the needed volume of PromoCell Medium in cell culture vessels. For equilibration, place the vessels in an incubator (37°C, 5% CO_2) for 30 minutes.

2. **Thaw the cells**
   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 into a water bath (37°C) just up to the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.

3. **Disinfect the vial and seed the cells**
   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. Open the vial and transfer the cells with a 2 ml serological pipette (not a micropipette) to a cell culture vessel containing the prewarmed medium from step 1 without resuspending.
   Note: Do not resuspend the cells at any time, since clumping may occur.



4. **Incubate the cells**
   Place the vessel in an incubator (37°C, 5% CO_2). For complete recovery, leave the cells untouched for at least 18 hours. Do not disrupt the flask during this recovery stage. Change the medium after 18–24 hours.
   Note: Handling of the cells before complete recovery results in clumping.

Use aseptic techniques and a laminar flow bench.

Subcultivation Protocol

1. **Harvest the cells**
   Harvest the cell suspension and determine the cell number. Spin down the cells for 10 minutes at 240 x g.


2. **Resuspend and reseed cells**
   Discard the supernatant (step 1), add 1 ml of the appropriate PromoCell Medium (step 2), and resuspend the cells by carefully pipetting up and down. Seed the cells according to the recommended seeding density in new cell culture vessels containing fresh PromoCell Medium prewarmed to 37°C. Place the vessels in an incubator (37°C, 5% CO_2).


Specifications

Product	Recommended Culture Media	Plating density
Human CD14+ Monocytes from Peripheral Blood (hMoCD14+-PB), single donor	C-28030	1 x 10^6 cells per ml

Related Products

Product	Size	Catalog Number
Mononuclear Cell Medium (Ready-to-use)	500 ml	C-28030
DC Generation Medium	250 ml	C-28050
DC Base Medium	250 ml	C-28053
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
hMNC-PB pooled Pellet	1 million cells per pellet	C-14099
hMoCD14+-PB single donor Pellet	1 million cells per pellet	C-14110
GM-CSF, human, recombinant	10 µg	C-60420
IL-4 CC, human, recombinant	5 µg	C-61401
M-CSF, human, recombinant	10 µg	C-60442
sRANKL, human, recombinant	10 µg	C-63200

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