Monocytes

PromoCell®

Instruction manual

Product	Size	Catalog number
Human CD14⁺ Monocytes from Peripheral Blood (hMoCD14⁺-PB), single donor	10 × 10⁶ 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) 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).

Our Human CD14⁺ Monocytes are isolated from fresh peripheral blood. After isolation of the ultra-pure mononuclear cell fraction by proprietary methods, the CD14+ Monocytes are isolated by positive selection.

Shortly after isolation, all our Human CD14⁺ Monocytes are cryopreserved using our proprietary, animal-component free, and protein-free cryopreservation medium (for details, please refer to the Certificate of Analysis). Each cryovial contains more than 10 million viable cells after thawing.

Quality control

Rigid quality control tests are performed for each lot of our CD14⁺ 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 contami nants (fungi, bacteria, and mycoplasma).

Intended use

PromoCell CD14⁺ Monocytes are for *in vitro* re search 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!

On delivery, immediately follow the protocol for cryopreserved cells (see page 2).

Protocol for cryopreserved cells

On delivery store the cryopreserved cells in liquid nitrogen or seed them immediately.

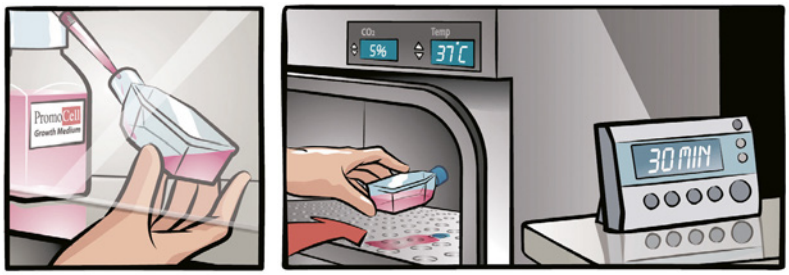
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

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

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 required volume of PromoCell Medium in cell culture vessels. To equilibrate, place the vessels in an incubator (37°C, 5% CO_2) for 30 minutes.



2

Thaw the cells

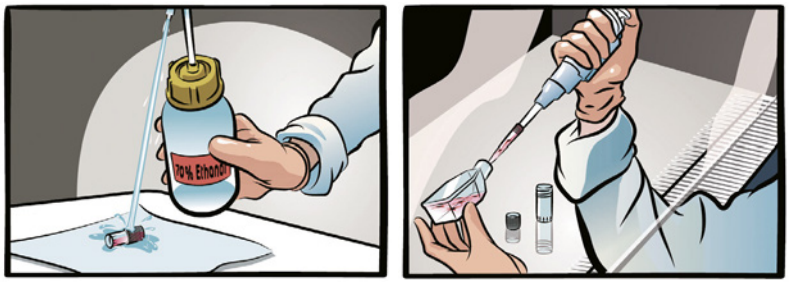
Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial into a water bath (37°C) up to the height of the screw cap 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 pre-warmed medium from step 1 without re-suspending.

Note: Do not re-suspend the cells at any time, as 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.

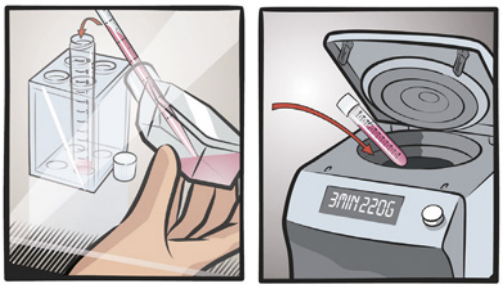
Subcultivation protocol

Use aseptic techniques and a laminar flow bench.

1

Harvest the cells

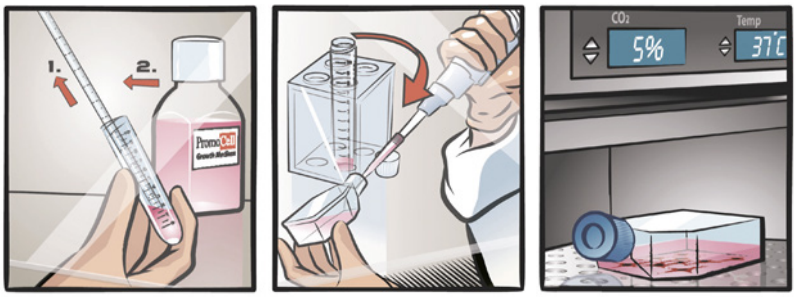
Harvest the cell suspension and determine the cell number. Spin down the cells for 10 minutes at 240 x g.



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 × 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 Plus	30 ml 125 ml	C-29920 C-29922
hMNC-PB pooled Pellet	1 million cells per pellet	C-14099
hMoCD14$^+$-PB single donor Pellet	1 million cells per pellet	C-14110

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 96 03 33 (toll free)
Fax: 0800 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

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