Mononuclear Cells

PromoCell

Instruction Manual

Product	Size	Catalog Number
Human Mononuclear Cells from Peripheral Blood (hMNC-PB), single donor, ultra-pure	25 x 10^6 cryopreserved cells	C-12907
Human Mononuclear Cells from Peripheral Blood (hMNC-PB), pooled, ultra-pure	25 x 10^6 cryopreserved cells	C-12908
Human Mononuclear Cells from Cord Blood (hMNC-CB), single donor, ultra-pure	25 x 10^6 cryopreserved cells	C-12901
Human Mononuclear Cells from Cord Blood (hMNC-CB), pooled, ultra-pure	25 x 10^6 cryopreserved cells	C-12904

Product Description

PromoCell Mononuclear Cells (MNC) represent the enriched lymphocyte and monocyte fraction of whole blood. They are isolated from umbilical cord blood and adult peripheral blood of healthy donors at PromoCell's cell culture facility. Mononuclear Cells from cord blood contain a relatively high percentage of primitive progenitor cells, whereas Mononuclear Cells from adult peripheral blood (hMNC-PB) contain large numbers of mature immune cells.

Note: When culturing pooled hMNC-PB, a bidirectional allogeneic mixed lymphocyte reaction (MLR) is likely to occur during the first 48 hours. This will result in cell activation, blast transformation and T-cell proliferation. Thus, these cells are preferably used for several types of infectious virus tests, including neutralization assays. For HIV-related research, prior $CD8^+$ T-cell depletion is recommended.

Freshly collected whole blood is carefully separated by optimized low-density gradient centrifugation, effectively removing the granulocytes. In order to avoid general cell damage, red blood cells are gently depleted by proprietary techniques instead of using classic osmotic lysis protocols. The obtained ultra-pure PromoCell Mononuclear Cells do not clump after thawing, and they exhibit superior viability and unchanged biological function.
Immediately after isolation, the freshly prepared Mononuclear Cells are cryopreserved using PromoCell´s proprietary, serum-free freezing medium, Cryo-SFM. Each cryo vial contains more than 25 million viable cells after thawing.

Quality Control

Rigid quality control tests are performed for each lot of PromoCell Mononuclear Cells.
They are routinely characterized by flow cytometry analyzing a series of cellular parameters, e.g. viability, cell size, granularity. PromoCell provides detailed information on the percentages of the major cellular sub-populations, i.e., the lymphocytes, the monocytes, and granulocytes, for each lot of Mononuclear Cells.
In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV and HCV, 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 Mononuclear Cells are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

Warning

Although tested negative for HIV-1, HIV-2, HBV, and HCV, 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.

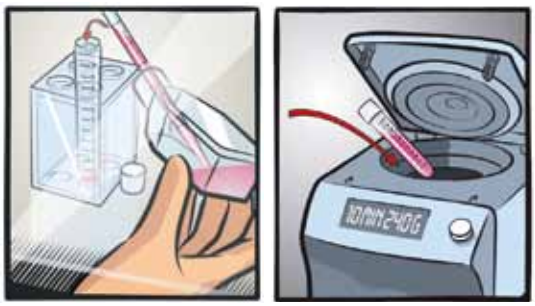
3. **Disinfect the vial and seed the cells**
   Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells with a 2 ml serological pipette (not a micropipette) to a cell culture 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.

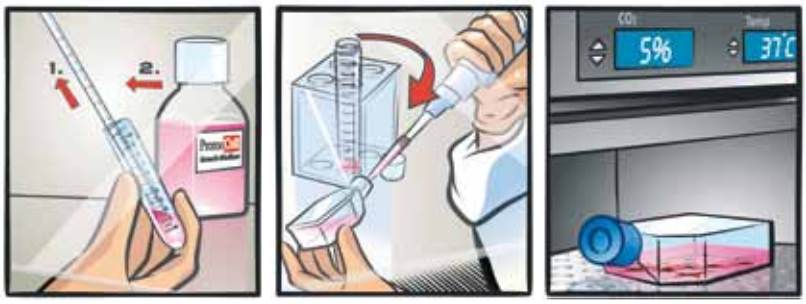
Use aseptic techniques and a laminar flow bench.

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 Medium	Recommended Differentiation Media	Plating density
Human Mononuclear Cells from Peripheral Blood (hMNC-PB), single donor, ultra-pure	C-28030		1 x 10^6 cells per ml
Human Mononuclear Cells from Peripheral Blood (hMNC-PB), pooled, ultra-pure	C-28030		1 x 10^6 cells per ml
Human Mononuclear Cells from Cord Blood (hMNC-CB), single donor, ultra-pure	C-28030 C-28021 C-39891	C-28020* C-28025*	1 x 10^6 cells per ml
Human Mononuclear Cells from Cord Blood (hMNC-CB), pooled, ultra-pure	C-28030 C-28021 C-39891	C-28020* C-28025*	1 x 10^6 cells per ml

* These differentiation media are applicable for CFU-assays for hematopoietic progenitor cells (LTC-IC assays) or "Hill-assays" for endothelial progenitor cells (Hill *et al.* 2003, N Engl J Med 348: 593-600).

Related Products

Product	Size	Catalog Number
Mononuclear Cell Medium (Ready-to-use)	500 ml	C-28030
Hematopoietic Progenitor Cell Expansion Medium DXF	500 ml	C-28021
Cytokine Mix E for HPC Expansion Medium DXF	1 ml (sufficient for 100 ml Medium) 5 ml (sufficient for 500 ml Medium)	C-39890 C-39891
Hematopoietic Progenitor Medium (Ready-to-use)	100 ml	C-28020
Endothelial Progenitor Medium (Ready-to-use)	100 ml	C-28025
Lymphocyte Separation Medium 1077	500 ml	C-44010
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
hMNC-CB single donor Pellet	1 million cells per pellet	C-14096
hMNC-CB pooled Pellet	1 million cells per pellet	C-14097
hMNC-PB single donor Pellet	1 million cells per pellet	C-14098
hMNC-PB pooled Pellet	1 million cells per pellet	C-14099
GM-CSF, human, recombinant	10 µg	C-60420
IL-4 CC, human, recombinant	5 µg	C-61401

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