

# Mononuclear Cells

## Instruction Manual

Product	Size	Catalog Number
Human Mononuclear Cells from Peripheral Blood (hMNC-PB), single donor, ultra-pure	25 × 10 <sup>6</sup> cryopreserved cells	C-12907
Human Mononuclear Cells from Cord Blood (hMNC-CB), single donor, ultra-pure	25 × 10 <sup>6</sup> cryopreserved cells	C-12901

### Product Description

Our Mononuclear Cells (MNCs) 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 our cell culture facility. MNCs from cord blood hMNC-CB contain a relatively high percentage of primitive progenitor cells, whereas MNCs from adult peripheral blood (hMNC-PB) contain large numbers of mature immune cells.

Freshly collected whole blood is carefully separated using optimized low-density gradient centrifugation, effectively removing the granulocytes. To avoid general cell damage, red blood cells are gently depleted using proprietary techniques instead of classic osmotic lysis protocols. The obtained ultra-pure PromoCell Mononuclear Cells do not clump after thawing, and exhibit superior viability and unchanged biological function.

Immediately after isolation, the freshly prepared Mononuclear Cells are cryopreserved

using PromoCell's proprietary, defined, animal-component free, and protein-free cryopreservation medium Cryo-SFM. Each cryovial contains more than 25 million viable cells after thawing.

### Quality Control

Rigid quality control tests are performed for each lot of our Mononuclear Cells. They are routinely characterized by flow cytometry analyzing a series of cellular parameters, e.g. viability, cell size, granularity. We provide detailed information on the percentages of major cellular sub-populations, i.e., lymphocytes, 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, 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](http://www.promocell.com/coa)

### Intended Use

PromoCell Mononuclear Cells are for in vitro research use only and are not suitable for diagnostic or therapeutic procedures.

### Warning

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

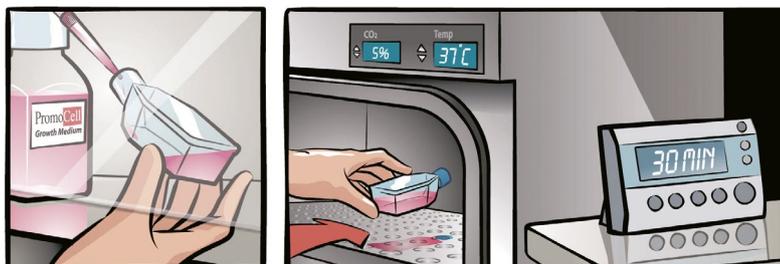
**Note:** Storage at  $-80^{\circ}\text{C}$  is not sufficient for cell preservation and causes irreversible cell damage.

*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 Growth Medium in cell culture vessels. To equilibrate, place the vessels in an incubator ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ) 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 re-tighten. Immerse the vial into a water bath ( $37^{\circ}\text{C}$ ) 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 ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ). 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.



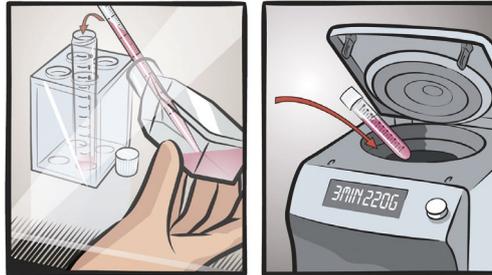
# Subcultivation Protocol

Use aseptic techniques and a laminar flow bench.

1

## Harvest the cells

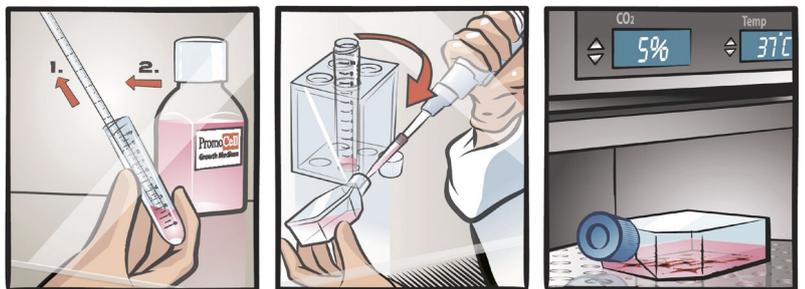
Harvest the cell suspension and determine the cell number. Spin down the cells for 10 minutes at 240 x g.



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<sub>2</sub>) and change the media every 2–3 days.



## Specifications

Product	Plating density
Human Mononuclear Cells from Peripheral Blood (hMNC-PB), single donor, ultra-pure	1 × 10 <sup>6</sup> cells per ml
Human Mononuclear Cells from Cord Blood (hMNC-CB), single donor, ultra-pure	1 × 10 <sup>6</sup> cells per ml

## Related Products

Product	Size	Catalog Number
Mononuclear Cell Medium (Ready-to-use)	500 ml	C-28030
Hematopoietic Progenitor Cell Expansion Medium XF	500 ml	C-28021
Cytokine Mix E for HPC Expansion Medium XF	1 ml (sufficient for 100 ml Medium) 5 ml (sufficient for 500 ml Medium)	C-39890 C-39891
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-PB single donor Pellet	1 million cells per pellet	C-14098

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