

Poietics™ CD14+ monocytes

Introduction

Monocytes are found in the circulating peripheral blood where they make up 10-20% of the mononuclear cells. They play an important role in host defense, both as circulating monocytes and by leaving the circulation and differentiating to tissue macrophages. Monocytes also differentiate into dendritic cells with potent antigen-presenting capability, *in vivo* and *in vitro*.

Macrophages themselves are difficult to isolate from tissue due to their phenotypic diversity in various tissues. Consequently, monocytes, which are the precursors to tissue macrophages, are a convenient source to use for macrophage research. Macrophages play key roles in cellular immunity, via phagocytosis and other signaling mechanisms, in addition to tissue remodeling that is essential to development and repair.

Monocytes can be isolated from peripheral blood using the cell surface marker CD14. CD14, the LPS receptor, is expressed on most monocytes, but not on other mononuclear cells in the blood. A second cell surface marker found on most monocytes is CD11b, a subunit of the integrin family.

Isolation

Poietics™ monocytes are isolated from the peripheral blood of screened, healthy donors. Peripheral blood is collected from the donors using apheresis. Monocytes are then isolated using positive immunomagnetic selection directed against CD14. Cells are then cryopreserved in three different sizes.

Characterization of cells

The purity of the population of cells is confirmed by flow cytometry using CD14 and CD11b.

Ordering information

Normal CD14+ monocytes:

2W-400A	≥40 million cells
2W-400B	≥20 million cells
2W-400C	≥10 million cells

Note: other sizes or configurations may be available upon request.

All other trademarks herein are marks of Lonza Group or its subsidiaries.

Media recommendations

The cryopreserved cells must be stored in liquid nitrogen until they are needed. Once thawed, the cells can be cultured in a variety of different conditions. To maintain the monocyte phenotype, serum-containing medium is recommended (10% FBS is generally recommended). M-CSF (at 10 ng/ml) can also be added. To produce osteoclasts, both soluble RANK ligand (66 ng/ml) and M-CSF (33 ng/ml) need to be added to serum-containing medium. To produce dendritic cells, a serum-free medium such as HPGM™ or LGM-3™ should be used, with the addition of GM-CSF (50 ng/ml) and IL-4 (50 ng/ml).

Research applications

- Phagocytic function and signaling
- Cellular immunity (HIV, cancer, sepsis)
- Antigen presentation
- Cytokine and chemokine action
- Immune cell differentiation
- Cell migration and adhesion
- Tissue remodeling
- Atherosclerosis
- Homeostasis
- Osteoporosis – see figure 1.

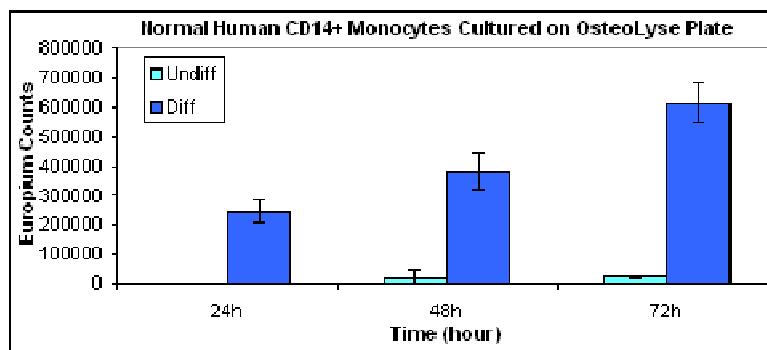


Figure 1. Poietics™ human CD14+ monocytes were thawed using the "Procedure for thawing Poietics™ cells" and directly seeded onto an OsteoLyse™ plate at 2.5×10^5 cells/well. Cells were cultured in OCP basal medium containing OCP growth medium SingleQuotes™ kit. Undifferentiated controls were cultured in medium with M-CSF only (final concentration of 33 ng/mL) and cells to be differentiated were cultured in medium containing both M-CSF and sRANKL (final

concentrations of 33 ng/mL and 66 ng/mL). Cells were cultured for 10 days at 37°C, in a humidified atmosphere of 5% CO₂. On **days 5 & 10**, the spent medium was carefully removed by pipetting and replaced with the appropriate fresh medium. Supernatants were sampled at 24, 48, and 72 hours post-medium change (days 11, 12, & 13). 10 µL of sample was added to 200 µL of room temperature fluorophore releasing agent in a black 96-well assay plate and gently mixed by pipetting. The fluorescence of each well in the assay plate is determined in a time-resolved fluorescence fluorimeter (e.g. a Wallac Victor, with excitation at 340 nm and emission at 615 nm) over a 400 µsecond time period after an initial delay of 400 µseconds.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing can not offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5th edition. If you require further information, please contact your site safety officer or scientific support.