Miltenyi Biotec

CytoBox Mo-DC
human

Order no. 130-100-842

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1. Description

Components CytoBox Mo-DC containing the following products:

Product	Content	Order no.
Human GM-CSF, premium grade	1×500 µg	130-093-867
Human IL-4, premium grade	2×100 µg	130-093-922

Biological activity The ED_{50} values of Human GM-CSF and Human IL-4 ED_{50} are <0.2 ng/mL* corresponding to a specific activity of $>5\times10^6$ IU/mg.

Primary structure Human GM-CSF: single, non-glycosylated polypeptide chain (127 amino acid residues).

Human IL-4: single, non-glycosylated polypeptide chain (130 amino acid residues).

Molecular mass Human GM-CSF: 14.5 kDa;
Human IL-4: 15.1 kDa.

Source Produced in *E. coli*.

Product format Lyophilized from a filtered (0.2 µm) buffer solution.

Stabilizer Mannitol and trehalose.

Purity >97% as determined by SDS-PAGE analysis.

Endotoxin level Low endotoxin (<0.1 EU/µg cytokine) as determined by Limulus Amebocyte Lysate (LAL) assay.

Storage Lyophilized Human GM-CSF and Human IL-4, premium grade should be stored at –20 °C. The expiration date is indicated on the vial label. Upon reconstitution aliquots should be stored at –20 °C or below. Avoid repeated freeze-thaw cycles.

*The ED_{50} values of GM-CSF and IL-4 are determined by proliferation assay using TF-1 cells according to Kitamura *et al.*[1]. The proliferation assays were calibrated with the international standards for human GM-CSF (NIBSC code 88/646) or IL-4 (NIBSC code 88/656) provided by the WHO/National Institute for Biological Standards and Control.

Reconstitution It is recommended to reconstitute lyophilized Human GM-CSF and Human IL-4 with deionized sterile-filtered water to a final concentration of 0.1–1.0 mg/mL in a minimal volume of 100 µL. Further dilutions should be prepared with 0.1% bovine serum albumin (BSA) or human serum albumin (HSA) in phosphate-buffered saline (PBS).

1.1 Background information

Dendritic cells (DCs) are the primary professional antigen-presenting cells of the immune system. They have a central function in initiation, programming, and regulation of antigen-specific immune responses[1]. *In vitro*, human DCs are often generated from peripheral blood monocytes using GM-CSF and IL-4.[2,3] These monocyte-derived DCs (Mo-DCs) have the characteristics of immature DCs. Mature Mo-DCs can be obtained by culturing Mo-DCs in the presence of microbial, pro-inflammatory, or T cell–derived stimuli[4]. Mo-DCs have been tested in a broad range of immunotherapy-based protocols, primarily in cancer research[5].

1.2 Applications

- Differentiation of human blood-derived monocytes into immature dendritic cells (Mo-DC) with a standardized protocol based on exact unit dosing, for maximal experimental reproducibility.

1.3 Reagent and instrument requirements

- Cell culture medium for Mo-DC culture containing FBS or human serum.
- PE buffer: Prepare a solution containing phosphate-buffered saline (PBS) and 2 mM EDTA, pH 7.4-7.6, sterile.

2. Protocol

2.1 Preparation of cell culture medium

In order to obtain maximal reproducibility for your Mo-DC differentiation experiments, it is recommended to always use the CytoBox Mo-DC cytokines at a defined unit dose (in IU/mL) for the Mo-DC culture. Lot-specific biological activities are stated on the Certificate of Analysis (CoA), provided by our technical support upon request. Follow the provided guidelines for optimal results:

1. Define final cytokine concentration in [IU/mL] for the cell culture. Recommended concentration for optimal Mo-DC differentiation is 1000 IU/mL of Human GM-CSF and 400 IU/mL of Human IL-4.

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140-004-186.02

2. Calculate the corresponding cytokine concentration in [ng/mL] for the Mo-DC culture.

$$\text{Example: GM-CSF final culture concentration in [ng/mL]} = \frac{1000\ \text{IU/mL}}{\text{lot-specific biological activity in [IU/mg]*}} \times 10^6$$

* Please, refer to corresponding CoA to obtain the exact value of lot-specific biological activity.

3. Dilute CytoBox Mo-DC cytokines in medium for Mo-DC culture at the calculated concentration in [ng/mL].

2.2 Preparation of cells

Highly pure $CD14^+$ monocytes can be isolated from peripheral blood mononuclear cells (PBMCs) using CD14 MicroBeads, human (# 130-050-201) or the Monocyte Isolation Kit II, human (# 130-091-153). For isolation of cells using the MACS® Technology, please refer to the respective data sheets.

2.3 Cell culture

1. Determine cell number.
2. Resuspend 10^6 cells per mL of complete medium containing CytoBox Mo-DC cytokines at a final concentration of 1000 IU/mL of Human GM-CSF and 400 IU/mL of Human IL-4 as described in chapter 2.1.
3. Plate cells in appropriate cell culture plate or flask.
4. Incubate cells for 3 days at 37 °C, 5% CO_2, and >95% humidity.
5. After 3 days, add complete medium containing CytoBox Mo-DC cytokines (Human GM-CSF, Human IL-4), 1 mL of medium per original 10^6 cells. Mix gently.

   ▲ **Note:** Do not remove supernatant prior to adding. Simply pipette appropriate amount of fresh prepared medium at 1× cytokine concentration.
6. Incubate cells for 4 days at 37 °C, 5% CO_2, and >95% humidity.
7. On day 7 harvest all cells. If cells adhere, remove cell culture medium containing floating cells and collect it in a tube. Immediately add PE buffer and incubate the flask at 37 °C. After 10 minutes harvest detached cells. Slightly adherent cells can be detached by gently tapping. Collect cells and transfer them in the same tube containing the floating cells.
8. Determine cell number of immature Mo-DCs.

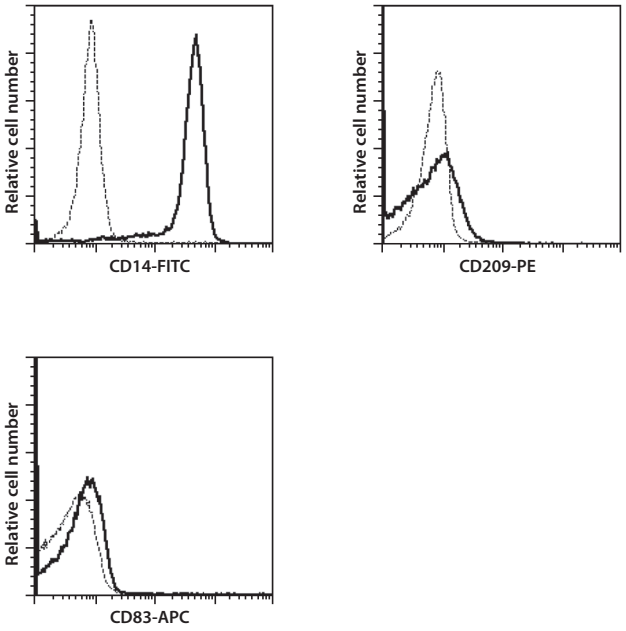
   ▲ **Note:** (Optional) Use Mo-DC Differentiation Inspector (# 130-093-567) for enumeration of immature Mo-DCs.

Maturation of DCs can be obtained with TLR ligands and pro-inflammatory cytokines, such as IL-6, TNF-α, IL-1β, and CD40-ligation that are available from Miltenyi Biotec. For further information please refer to www.miltenyibiotec.com.

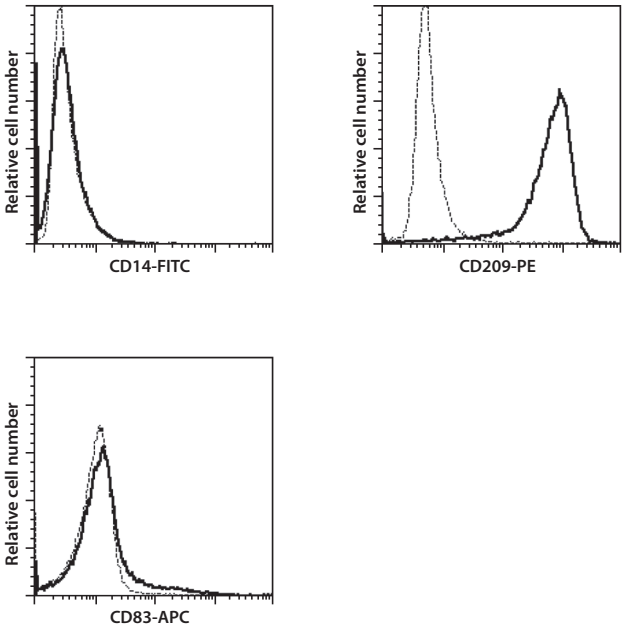
3. Example of Mo-DC generation using the CytoBox Mo-DC

Flow cytometric analysis of *in vitro* generated monocyte-derived dendritic cells (Mo-DCs) using the Mo-DC Differentiation Inspector. Immature Mo-DCs were generated by culturing purified $CD14^+$ monocytes (10^6/mL) in the presence of GM-CSF (1000 IU/mL) and IL-4 (400 IU/mL) for 7 days as described in chapter 2.

Monocytes



Immature monocyte-derived dendritic cells (Mo-DCs)



4. References

1. Banchereau, J. and Steinman, R. M. (1998) Dendritic cells and the control of immunity. Nature 392: 245–252.
2. Sallusto, F. and Lanzavecchia, A. (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. J. Exp. Med. 179: 1109.
3. Pickl, W. F. *et al.* (1996) Molecular and functional characteristics of dendritic cells generated from highly purified $CD14^+$ peripheral blood monocytes. J. Immunol. 157: 3850–3859.
4. Castiello, L. *et al.* (2011) Monocyte-derived DC maturation strategies and related pathways: a transcriptional view. Cancer Immunol. Immunother. 60: 457–466.
5. Palucka, K., and Banchereau, J. (2012). Cancer immunotherapy via dendritic cells. Nat. Rev. Cancer. 12: 265–277.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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