Miltenyi Biotec

Mo-DC Differentiation Medium
human

Order no. 130-094-812

Contents

1. Description
   - 1.1 Background information
   - 1.2 Applications
   - 1.3 Reagent and instrument requirements
2. Protocol
3. References

1. Description

This product is for research use only.

Components	400 mL Mo-DC Differentiation Medium, human: with fetal bovine serum (FBS) with RPMI 1640 with L-glutamine with IL-4 with GM-CSF
Quality control	Satisfactory differentiation of Mo-DC from monocytes isolated from human PBMCs.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The Mo-DC Differentiation Medium, human is based on Rosewell Park Memorial Institute (RPMI) 1640 Medium and contains L-Glutamine, Interleukin-4 (IL-4), and granulocyte macrophage colony-stimulating factor (GM-CSF) and is also part of the Mo-DC Generation Toolbox, human. Large numbers of Mo-DCs can be generated *ex vivo* from purified $CD14^+$ monocytes.[1–6] Immature monocyte-derived dendritic cells (Mo-DCs) can easily be differentiated from monocytes with a lot-to-lot consistent standardized protocol.

1.2 Applications

- Differentiation of Mo-DCs.

1.3 Reagent and instrument requirements

- Tissue culture flask (T-75, 75 cm^2)
- (Optional) PE buffer

2. Protocol

Cell culture – Mo-DC differentiation

1. Determine cell number.
2. Resuspend 10^7 cells per 10 mL of Mo-DC Differentiation Medium and mix well.
3. Plate cells in a tissue culture flask (T-75 , 75 cm^2).
4. Incubate cells for 3 days at 37 °C, 5% CO_2, and >95% humidity.
5. Add 10 mL of Mo-DC Differentiation Medium to feed cells after 3 days and mix well.

   ▲ **Note:** Do not remove used Mo-DC Differentiation Medium prior to adding, simply pipette appropriate amount of fresh Mo-DC Differentiation Medium.
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 use cell scraper or release cells by adding PE buffer after removal of cell culture medium and incubate the flask for 10 minutes at 37 °C. Do not shake to release adherent cells.
8. Determine cell number of immature Mo-DCs.

   ▲ **Note:** (Optional) Using Mo-DC Differentiation Inspector for enumeration of immature Mo-DCs.

   ▲ **Note:** (Optional) For the following maturation of Mo-DCs please refer to the data sheet of the Mo-DC Generation Toolbox, human.

3. References

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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 alpha. J. Exp. Med. 179: 1109–1118.
3. Dauer, M. *et al.* (2003) Mature dendritic cells derived from human monocytes within 48 hours: a novel strategy for dendritic cell differentiation from blood precursors. J. Immunol. 170: 4069–4076.
4. Zhang, R. *et al.* (2006) C-reactive protein impairs human $CD14^+$ monocyte-derived dendritic cell differentiation, maturation and function. Eur. J. Immunol. 36: 2993–3006.
5. Sanarico, N. *et al.* (2006) Human monocyte-derived dendritic cells differentiated in the presence of IL-2 produce proinflammatory cytokines and prime Th1 immune response. J. Leukoc. Biol. 80: 555–562.
6. Assier, E. *et al.* (2007) TLR7/8 agonists impair monocyte-derived dendritic cell differentiation and maturation. J. Leukoc. Biol. 81: 221–228.

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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