Mar 13, 2019 Version 1

Differentiation of dendritic cells from human monocytes V.1

DOI
dx.doi.org/10.17504/protocols.io.xjrfkm6

Girija Goyal[1]

[1]Wyss Institute for Biologically Inspired Engineering

Girija Goyal

DOI: https://dx.doi.org/10.17504/protocols.io.xjrfkm6

Protocol Citation: Girija Goyal 2019. Differentiation of dendritic cells from human monocytes. **protocols.io https://dx.doi.org/10.17504/protocols.io.xjrfkm6**

Protocol status: Working
We use this protocol and it's working

Created: January 30, 2019

Last Modified: March 13, 2019

Protocol Integer ID: 19793

Keywords: dendritic cells from human monocyte, differentiation of dendritic cell, dendritic cell, dendritic cell lineage, human monocytes this protocol, human monocyte, dc, cell, considered classical dc, classical dc

Abstract

This protocol describes the steps conventionally used to differentiate dendritic cells from human monocytes isolated from fresh whole blood or leukopaks. While these DC mimic many of the functions of the dendritic cell lineage, they are not considered classical DC which are a different lineage and very rare. By day 4 CD1c+ cells are emerging with a reduction in CD14 positivity

Guidelines

1. The amount of cytokines needed is very variable from laboratory to laboratory and from manufacturer to manufacturer. The cytokines used in this protocol were obtained from Miltenyi Biotec. If cytokines are ordered from a different source, the protocol should be reoptimized.
2. Only 50% of the media should be replaced. Complete replacement can retard the differentiation.
3. Fresh cytokines need to be added at least every 3, preferably every 2 days.
4. We get better results using isolated monocytes directly rather than plating whole PBMC and waiting for the monocytes to adhere.
5. Publications show (and we have confirmed) the induction of dendritic cell markers by day 5.
6. If media starts going yellow every day, then the dendritic cells need to be collected. Collected dendritic cells can be frozen down for later use. Some monocytic progenitors are also left behind in the well and will keep producing dendritic cells if cultured further.

Troubleshooting

Safety warnings

BL1 safety procedures should be followed.

Plate monocytes

1 Plate monocytes in a 24 well plate with 1 million monocytes/well in 1 ml of RPMI+400ng/ml GM-CSF and 250ng/ml IL4.

First media change

2 2 days later (maximum 3 days), assess if there are any non-adherent, small cells floating around. These might be neutrophils or lymphocytes. If the number of these cells is high, it can impact the differentiation of DC. To remove non-adherent cell, gently aspirate all the media in the well and add fresh cytokine containing media. If the non-adherent cells are very few in number, proceed to step 3.

Subsequent media changes

3 If culturing past day 5, only replace half of the media by gently aspirating from the top and then adding fresh cytokine containing media.

Dendritic cell collection

4 Collect dendritic cells on day 5 or later by pipetting multiple times around the well to remove cells and then collecting the suspended cells.