

FAST, EASY, GENTLE!

pluriBead®

Application Example

**Monocyte isolation with pluriBead®
for maturation of dendritic cells**



pluriSelect products in this flyer

CD14 S-pluriBead® anti-hu	11-01400-10
CD14 M-pluriBead® anti-hu	11-01400-20
S-pluriBead® Mini Reagent Kit	70-50010-11
M-pluriBead® Mini Reagent Kit	70-50010-21
pluriPlix® 3-in-1 Mixer	50-01010-80

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Monocyte isolation with pluriBead® for maturation of dendritic cells

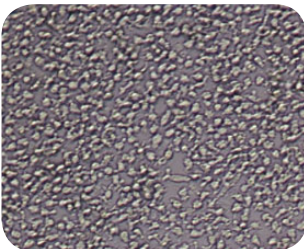
Sample volume	10 ml buffy coat
Isolation method	280 µl CD14 S-pluriBead® anti-Hu or 300 µl CD14 M-pluriBead® anti-Hu
Yield	~6 x 10 ⁶ monocytes with S-pluriBead® ~12 x 10 ⁶ monocytes with M-pluriBead®
Vitality	>92% (trypan blue staining)
Purity	~97%

Monocyte isolation, differentiation and stimulation protocol

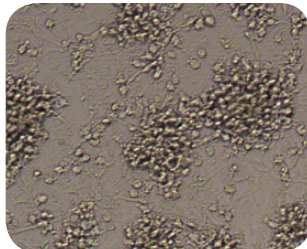
To reduce soluble CD14 wash sample material twice with the provided Washing Buffer. Add CD14 pluriBead® for isolation of monocytes into the sample tube. Incubate on a pluriPlix® or rolling mixer for 20 minutes. Detach the CD14 cells from pluriBead®.

Resuspend the cells in 1 ml of RPMI 1640 medium (plus 10% FCS and 1x Pen/Strep). Determine the cell number. Use 1 million cells per well for the cultivation of monocyte in a 24-well cell culture plate. Incubate with 1 ml monocyte culture medium (RPMI 1640 plus 10% FCS, 1x Pen/Strep, 2,000 U/ml GM-CSF and 200 U/ml IL-4) at 37°C and 5% carbon dioxide.

Remove the medium and add 1 ml fresh monocyte culture medium after 24 hours. Culture the cells for 4 more days. Stimulate the cells with 100 ng/ml LPS for another 24 hours (after a total of 5 days). Remove the activated cells with trypsin. Analyze the maturation rate to dendritic cells from monocytes by fluorescent analysis of CD1a+, CD14+ and CD83+.

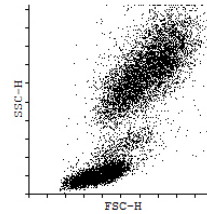


CD14+ cells after isolation in culture

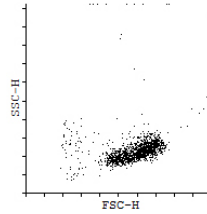


Maturation of monocytes to dendritic cells after 5 days of culture with GM-CSF/IL-4 and stimulation with LPS

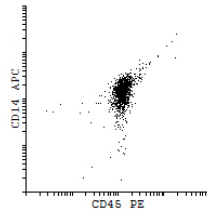
Fluorescent analysis of monocytes



Human sample material before separation (FSC SSC)

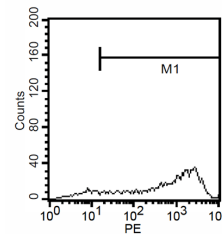


Isolated CD14+ cells (FSC SSC)

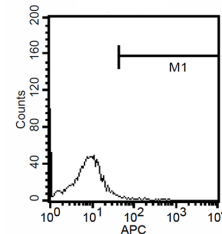


Isolated CD14+ cells (CD45 PE CD14 APC)

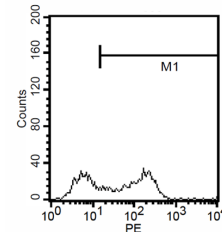
Fluorescent analysis of dendritic cells



Fluorescent analysis of CD1a+ on mature dendritic cells



Fluorescent analysis of CD14+ on mature dendritic cells



Fluorescent analysis of CD83+ on mature dendritic cells