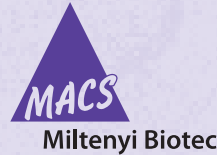


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Blood Dendritic Cell Enumeration Kit human

For 50 tests

Order no. 130-091-086



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1. Description

1.1 Peripheral blood dendritic cells

Two subsets of dendritic cells (DCs) were originally identified in peripheral blood, myeloid DCs (MDCs), and plasmacytoid DCs (PDCs).¹ PDCs are also known as plasmacytoid T cells², plasmacytoid monocytes³, IFN- α / β -producing cells (IPC)⁴⁻¹¹, type 2 pre-DCs (pDC2)¹²⁻¹⁵, lymphoid DCs¹⁶, CD11c⁺ DCs¹, and CD123⁺ DCs¹⁷. MDCs are also designated as type 1 DCs (DC1)¹⁸ and CD11c⁺ DCs¹. MDCs and PDCs differ widely in many respects. They express different patterns of pathogen recognition receptors¹⁹⁻²¹ and accordingly respond to different microbial antigens^{15,19,20}. They express different cytokine receptors and as a result respond to different cytokines.^{22,23} They express different cytokines, even in response to identical stimuli.²⁴ Finally, they differ widely in the capacity to migrate to chemotactic stimuli, although the pattern of expressed chemokine receptors, with the exception of CCR7, is similar.²⁵ These differences point to distinct roles of MDCs and PDCs in the induction and regulation of the immune response.

Recently, it was demonstrated that MDCs do not represent a homogeneous population but rather consist of two subsets.^{26,27} Most MDCs are capable of differentiating into Langerhans cells (LCs) when cultured in the presence of GM-CSF, IL-4, and TGF- β 1, however, a minor fraction lacks this capability.²⁶ The fraction which is capable of differentiating into LCs (MDC1s) differs from the fraction which lacks this capability (MDC2s) in that it expresses CD2 and Fc receptors (CD32, CD64, and Fc ϵ RI).

In the past, the identification of blood DCs has been difficult due to the lack of specific surface antigens. Commonly, identification of DCs in blood is based on a multitude of immunophenotypic criteria, such as the absence of a panel of leukocyte lineage-specific antigens (CD3, CD14, CD16, CD19, CD20, and CD56) and the presence of HLA-DR. MDCs and PDCs are often distinguished among HLA-DR⁺ lineage⁻ DCs based on expression of CD11c on MDCs and CD123 on PDCs. This approach requires staining with nine monoclonal antibodies conjugated to four fluorochromes and provides no possibility of excluding dead cells and discriminating MDC1s from MDC2s.

Recently, Dzionek *et al.*²⁷ described monoclonal antibodies against novel markers of DCs in blood: (1) CD303 (BDCA-2) and CD304 (BDCA-4/Neuropilin-1) for PDCs, (2) CD1c (BDCA-1) for MDC1s, and (3) CD141 (BDCA-3) for MDC2s. In blood, expression of BDCA-2 and BDCA-4 is strictly confined to PDCs.²⁷⁻³⁰ CD1c (BDCA-1) is expressed on a considerable proportion of CD19⁺ B cells and on MDC1s.^{27,28} BDCA-3 is expressed at high levels only on MDC2s but at low levels also on PDCs, MDC1s, subsets of monocytes, and on granulocytes.^{27,28}

Using these novel markers, we have developed the Blood Dendritic Cell Enumeration Kit for easy counting of DCs in blood by flow cytometry. PDCs and the two subsets of MDCs are identified via staining with Anti-BDCA-2-FITC, CD1c (Anti-BDCA-1)-PE, and Anti-BDCA-3-APC, respectively. B cells, which also express CD1c (BDCA-1), and monocytes, which express BDCA-3 at a low level, are excluded from the analysis based on staining with CD19-PE-Cy5 and CD14-PE-Cy5, respectively. Dead cells are excluded via staining with a cell-impermeant dye that, after photolysis, binds covalently to nucleic acids in cells with compromised

membranes. After staining and erythrocyte lysis, cells are fixed and subsequently analyzed by three-color (FITC, PE, and PE-Cy5) or four-color (FITC, PE, PE-Cy5, and APC) flow cytometry. Only four-color flow cytometry allows enumeration of MDC2s.

Applications

The kit has been developed for the identification and enumeration of DCs and DC subsets in whole blood or peripheral blood mononuclear cells (PBMCs). Changes in the number of peripheral blood PDCs and/or MDCs have been observed, for example:

- in patients with systemic lupus erythematosus (SLE);³¹
- in HIV-infected AIDS patients;³²⁻³⁵
- in healthy human volunteers after administration of either Flt3-Ligand or G-CSF;^{36,37}
- in healthy infants and children with increasing age;³⁸ and
- in patients with chronic viral hepatitis.³⁹

1.2 Kit size and storage

Kit size	50 tests
Test size	One test corresponds to the analysis of two samples of either 300 µL whole anticoagulated blood or 1×10 ⁶ PBMCs.
Storage	Store reagents protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the box label.

1.3 Assay principle

The assay can be performed either with whole blood or with PBMCs. For one test, two samples of 300 µL whole blood or two samples of 1×10⁶ PBMCs are needed. PDCs, MDC1s, and MDC2s are detected in one sample by staining with CD303 (BDCA-2)-FITC, CD1c (BDCA-1)-PE, and CD141 (BDCA-3)-APC, respectively. The other sample is stained with FITC-, PE-, and APC-conjugated isotype control monoclonal antibodies. Both samples are simultaneously stained with CD19-PE-Cy5 and CD14-PE-Cy5 for exclusion of B cells and monocytes. All monoclonal antibodies needed for staining of each sample are supplied as pre-mixed cocktails (**Anti-BDCA Cocktail** and **Control Cocktail**). Simultaneously to antibody staining, samples are stained with a fluorescent cell-impermeant dye (**Dead Cell Discriminator**) which, after illumination with visible light, binds covalently and irreversibly to nucleic acids of dead cells. After cell staining, erythrocytes are lysed with the **Red Blood Cell Lysis Solution**. Cells are then washed and fixed using formaldehyde (**Fix Solution**), and **Discriminator Stop Reagent** is added to the samples for optimal dead cell discrimination even after prolonged storage. Finally, cells are analyzed by three-color (FITC, PE, PE-Cy5) flow cytometry for enumeration of PDCs and MDC1s, or by four-color (FITC, PE, PE-Cy5, APC) flow cytometry for enumeration of PDCs, MDC1s, and MDC2s.

1.4 Kit components

1 mL Anti-BDCA Cocktail

Cocktail of fluorochrome-conjugated monoclonal antibodies: CD1c (BDCA-1)-PE (clone: AD5-8E7, isotype: mouse IgG2a), CD303 (BDCA-2)-FITC (clone: AC144, isotype: mouse IgG1), CD141 (BDCA-3)-APC (clone: AD5-14H12, isotype: mouse IgG1), CD14-PE-Cy5 (isotype: mouse IgG2a), and CD19-PE-Cy5 (isotype: mouse IgG1), supplied in a solution containing stabilizer and 0.05% sodium azide.

1 mL Control Cocktail

Cocktail of fluorochrome-conjugated monoclonal antibodies: Mouse IgG2a-PE, Mouse IgG1-FITC, Mouse IgG1-APC, CD14-PE-Cy5 (isotype: mouse IgG2a), and CD19-PE-Cy5 (isotype: mouse IgG1), supplied in a solution containing stabilizer and 0.05% sodium azide.

1 mL Dead Cell Discriminator

Fluorescent photolytic dye dissolved in PBS.

50 mL Red Blood Cell Lysis Solution (10×)

25 mL Fix Solution

3.7% formaldehyde in PBS (EU Hazard Classification: Xn harmful R40/20/21/22-43).

0.5 mL Discriminator Stop Reagent

1.5 Description of kit components

Anti-BDCA Cocktail

The Anti-BDCA Cocktail is a pre-mixed cocktail which includes all antibodies needed for direct identification of PDCs, MDC1s, and MDC2s by flow cytometry. The DC-specific antibodies CD303 (BDCA-2), CD1c (BDCA-1), and CD141 (BDCA-3) are conjugated to different fluorochromes and are detected in separate fluorescence channels of the flow cytometer. Apart from the DC-specific antibodies, the Anti-BDCA Cocktail contains monoclonal antibodies directed against CD19 for exclusion of B cells and CD14 for exclusion of monocytes. B cells and monocytes are excluded from the analysis as a subpopulation of B cells expresses CD1c (BDCA-1), and monocytes express CD141 (BDCA-3) at a low level. Both antibodies are conjugated to PE-Cy5.

Components of the Anti-BDCA Cocktail

Antibody	Fluorochrome	Specificity
CD303 (BDCA-2)	FITC	PDCs
CD1c (BDCA-1)	PE	MDC1s
CD14	PE-Cy5	Monocytes
CD19	PE-Cy5	B cells
CD141 (BDCA-3)	APC	MDC2s

Fluorescein (FITC), Phycoerythrin (PE), Phycoerythrin-Cyanine 5 (PE-Cy5), Allophycocyanin (APC)

Three-Color
Four-Color

Control Cocktail

Apart from monoclonal antibodies directed against CD14 and CD19, the Control Cocktail contains monoclonal antibodies which match the isotype of the DC-specific antibodies in the Anti-BDCA Cocktail but have irrelevant specificities (isotype control). Thereby, the frequency of non-specific cell staining, for example, via Fc receptors, is determined. For reliable enumeration of dendritic cells, an isotype control sample is always included.

Components of the Control Cocktail

Antibody	Fluorochrome	Specificity
Mouse IgG1	FITC	Isotype control
Mouse IgG2a	PE	Isotype control
CD14	PE-Cy5	Monocytes
CD19	PE-Cy5	B cells
Mouse IgG1	APC	Isotype control

Three-Color
Four-Color

Dead Cell Discriminator and Discriminator Stop Reagent

The Dead Cell Discriminator is a photoaffinity fluorescent dye which allows discrimination of dead cells in combination with cell fixation. This is particularly useful when working with hazardous samples. The membrane-impermeant dye infiltrates selectively dead cells due to their damaged membranes. In the cell, it binds covalently and irreversibly to nucleic acids after exposure to visible light. Cells are, thus, incubated under a 60 W light bulb. Afterwards, they are washed, fixed, and analyzed. Finally, the Discriminator Stop Reagent is added, which allows optimal

dead cell discrimination even after prolonged storage (24 hours) of the fixed cells at 2–8 °C.

The Dead Cell Discriminator is excited by a laser emitting light at 488 nm and has a fluorescence emission maximum at 625 nm. The fluorescence signal is very similar to that of propidium iodide and accordingly does not need fluorescent compensation upon flow cytometric analysis.

Red Blood Cell Lysis Solution

Upon incubation of the cells in 1× Red Blood Cell Lysis Solution erythrocytes are selectively lysed.

Fix Solution

After adding the Fix Solution, cells can be stored at 2–8 °C in the dark for up to 24 hours prior to analysis without considerable changes in the cell morphology.

1.6 Starting material

The assay is designed for analysis of whole blood or PBMCs. For best results use blood within 24 hours after collection.

Whole blood Whole blood has to be supplemented with an anticoagulant, e.g., heparin, citrate, acid citrate dextrose (ACD), or citrate phosphate dextrose (CPD). Whole blood should be stored at room temperature until analysis.

PBMCs

If PBMCs are used, Fc receptor-mediated antibody staining must be blocked, e.g., by adding FcR Blocking Reagent (# 130-059-901) and/or by supplementing of the buffer with 10% autologous serum. PBMCs should be stored in buffer at 2–8 °C until analysis.

1.7 Additional reagents and instruments required

- Anticoagulant
- Buffer: phosphate buffered saline (PBS), pH 7.2, containing 0.5% bovine serum albumin (BSA) and 0.01% sodium azide
- Double distilled water (ddH₂O)
- Two 12×75 mm polystyrene tubes (volume 4.5 mL) with caps per test, which are optimally compatible to the flow cytometer uptake port
- Ice
- 60 W light bulb
- Micropipettes with tips
- Centrifuge (2–8 °C)
- Vortex mixer
- Single- or dual-laser flow cytometer and software (Chapter 3 and Appendix)
- Equipment for assessing white blood cell counts
- FcR Blocking Reagent (# 130-059-901) for analysis of PBMCs

2. Staining protocols

2.1 Dendritic cell enumeration in whole blood

Before starting:

- ▲ Prepare 10 mL buffer: phosphate buffered saline, 0.5% bovine serum albumin, 0.01% sodium azide.
- ▲ Dilute 1 mL **10× Red Blood Cell Lysis Solution** by adding 9 mL double distilled water. Do not use deionized water!
- ▲ Determine the total number of leukocytes per mL of whole blood.

Two samples



1. Take two 12×75 mm tubes and transfer 300 μ L of whole anticoagulated blood into each tube .



2. Add 20 μ L **Anti-BDCA Cocktail** to one sample and 20 μ L **Control Cocktail** to the other sample.



3. Add 10 μ L **Dead Cell Discriminator** to each sample.



4. Mix gently and incubate each tube for 10 minutes in a **horizontal** position on ice under a 60 W light bulb (distance 3–5 cm).



5. Add 4 mL **1× Red Blood Cell Lysis Solution** to each sample.



6. Mix gently and incubate at room temperature in the dark for 10 minutes.



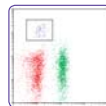
7. Spin cells down at 300×g for 5 minutes, aspirate supernatant completely, and resuspend the cells in 4 mL of buffer.

2x

8. Spin cells down at 300×g for 5 minutes, aspirate supernatant completely, and resuspend the cells in 300 μ L of buffer.



9. Add 150 μ L **Fix Solution** and 5 μ L **Discriminator Stop Reagent** to each sample.



10. Analyze samples by flow cytometry (Chapters 3 and 4). Samples can be stored at 2–8 °C in the dark for up to 24 hours prior to analysis.

2.2 Dendritic cell enumeration in PBMCs

Before starting:

- ▲ Prepare 10 mL buffer: phosphate buffered saline, 0.5% bovine serum albumin, 0.01% sodium azide.
- ▲ Fc receptor-mediated antibody staining must be blocked, e.g., by adding FcR Blocking Reagent (step 2) and/or by supplementing the buffer with 10% autologous serum.
- ▲ Determine the total number of leukocytes per mL PBMCs.

Two samples



1. Take two 12×75 mm tubes and transfer 1×10^6 PBMCs in 240 μ L of buffer into each tube.



2. Add 60 μ L **FcR Blocking Reagent** (# 130-059-901) to each sample.



3. Add 20 μ L **Anti-BDCA Cocktail** to one sample and 20 μ L **Control Cocktail** to the other sample.



4. Add 10 μ L **Dead Cell Discriminator** to each sample.



5. Mix gently and incubate each tube for 10 minutes in a **horizontal** position on ice under a 60 W light bulb (distance 3–5 cm).



6. Add 4 mL of buffer.



7. Spin cells down at 300×g for 5 minutes, aspirate supernatant completely, and resuspend the cells in 300 μ L of buffer.



8. Add 150 μ L **Fix Solution** and 5 μ L **Discriminator Stop Reagent** to each sample.



9. Analyze samples by flow cytometry (Chapters 3 and 4). Samples can be stored at 2–8 °C in the dark for up to 24 hours prior to analysis.

3. Flow cytometric data acquisition

Instrument configuration for multi-color immunofluorescence analysis

Three-color flow cytometry

PDCs and MDC1s can be simultaneously enumerated by performing three-color flow cytometry on the MACSQuant™ Analyzer. In addition to MACSQuant Analyzer, other flow cytometers with a 488 nm laser and appropriate filter sets for detection of FITC, PE, and PE-Cy5 can also be used. For a list of suitable flow cytometers capable of performing three- and four-color flow cytometry, see Appendix B.

Four-color flow cytometry

For simultaneous identification of PDCs, MDC1s, and MDC2s, the MACSQuant Analyzer or another flow cytometer with two lasers (a 488 nm laser and, for example, a 635 nm laser) and appropriate filter sets for detection of FITC, PE, PE-Cy5, and APC are required (see also Appendix A).

Acquisition and analysis software

For data acquisition and data analysis, commercially available software can be used, for example, MACSQuantify.

Instrument setting

Choose a flow cytometer instrument setting for standard three- or four-color cell analysis of human blood leukocytes. A proper instrument setting can be reached by adjusting parameters with separate FITC-, PE-, PE-Cy5-, and APC-conjugated antibodies or by using commercially available fluorescence reference standards for three- or four-color analysis of appropriate fluorochromes.

- Exclude debris from data acquisition by an appropriate value for the threshold on forward scatter.
- Proper fluorescence compensation is important to obtain accurate results.

Creation of dot plots

For data acquisition create following dot plots:

- forward scatter (FSC) vs. side scatter (SSC)
- SSC vs. PE-Cy5, Dead Cell Discriminator
- FITC vs. PE
- FITC vs. APC

Parameter Description

Anti-BDCA sample	Control sample	Parameter	Specificity
		FSC	
		SSC	
CD303 (BDCA-2)	Mouse IgG1	FITC	PDCs/control
CD11c (BDCA-1)	Mouse IgG2a	PE	MDC1s/control
DCD	DCD	DCD	dead cells
CD14	CD14	PE-Cy5	monocytes
CD19	CD19	PE-Cy5	B cells
CD141 (BDCA-3)	Mouse IgG1	APC	MDC2s/control
Dead Cell Discriminator (DCD)			



Data acquisition

- Since DCs are generally present at a low frequency in blood, acquire as many events from the sample as possible.
- Typically, MDC2s are present at a frequency of about 0.01% among peripheral blood leukocytes (see Appendix D). Thus, in order to detect 100 MDC2s, at least 1,000,000 events must be acquired. If too few events are acquired and/or MDC2s are present at a frequency below 0.01%, MDC2s may not be detected at all.

4. Flow cytometric analysis (three- or four-color)

▲ The gating strategy below describes the flow cytometric analysis of a whole blood sample using MACSQuantify. Proceed accordingly, if another software is used.

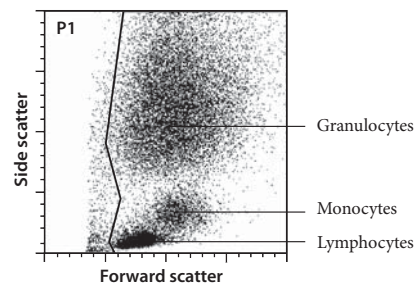
▲ Perform the analysis of the sample stained with the Anti-BDCA Cocktail in the same way as the analysis of the sample stained with the Control Cocktail.

▲ Gating strategies for whole blood and PBMCs are identical.

1. Discrimination of debris and platelets

Create a FSC/SSC dot plot (no gate).

Draw region P1 to exclude debris and platelets.



(Control sample not shown.)

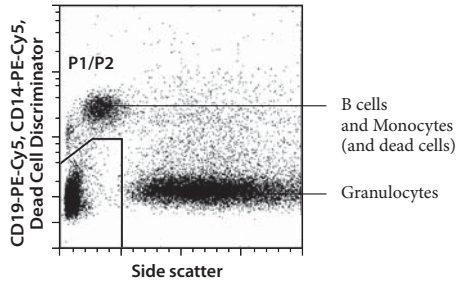
2. Exclusion of B cells, monocytes, granulocytes (neutrophils and eosinophils), and dead cells

Create a SSC vs. PE-Cy5/DCD (SSC/CD19-PE-Cy5, CD14-PE-Cy5, Dead Cell Discriminator) dot plot gated on P1.

Draw region P2 exactly as shown on the next page to exclude B cells, monocytes, granulocytes (neutrophils and eosinophils), and dead cells.

Generate a list statistic of the dot plot for later calculation (Chapter 4, step 5) of DCs frequencies within leukocytes.

Gated on P1



(Control sample not shown.)

Because DCs have a SSC value between lymphocytes and granulocytes, draw P2 closely to the edge of the granulocyte population.

Because some CD1c (BDCA-1)⁺ MDC1s express at low levels CD14, create P2 in that way that events with a low PE-Cy5 signal are included and events with a high PE-Cy5 signal (CD19⁺ B cells, CD14⁺ monocytes, and dead cells) are excluded.

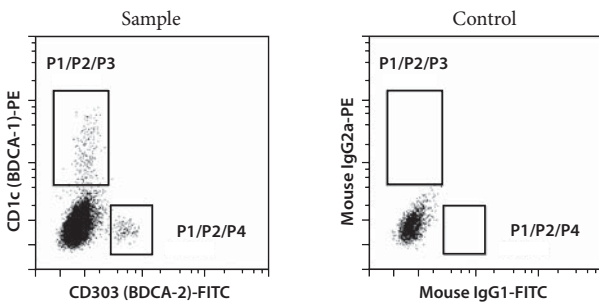
Some blood leukocytes, including MDCs, tend to become non-specifically stained by PE-Cy5-conjugated monoclonal antibodies. Also for this reason, P2 should include events (except granulocytes) with a low signal.

3. Identification of PDCs and MDC1s

Create a FITC/PE (CD303 (BDCA-2)-FITC/CD1c (BDCA-1)-PE) dot plot gated on P1/P2.

Draw region P3 to define CD1c (BDCA-1)⁺ MDC1s and region P4 to define CD303 (BDCA-2)⁺ PDCs exactly as shown here.

Gated on P1/P2



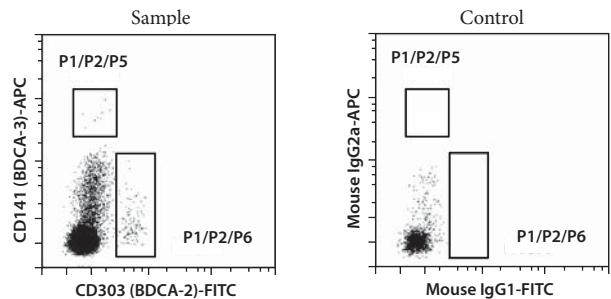
▲ If only a three-color analysis is performed, continue with Chapter 4, step 5 of the analysis procedure.

4. Identification of MDC2s

Create a FITC/APC (CD303 (BDCA-2)-FITC/CD141 (BDCA-3)-APC) dot plot gated on P1/P2.

Draw region P5 exactly as shown here to define CD141 (BDCA-3)⁺ MDC2s.

Gated on P1/ P2



CD141 (BDCA-3) is also expressed at much lower levels on monocytes, granulocytes, PDCs, and MDC1s. Therefore create P5 in a way that only events with a high APC signal (CD141 (BDCA-3)-APC) are included.

Some blood leukocytes, including MDCs, tend to become non-specifically stained by PE-Cy5-conjugated monoclonal antibodies. For this reason, the isotype control staining might reveal quite a few events with low APC signal.

5. Determination of DC subset frequencies

Obtain the frequency of each DC subset in whole blood and the frequencies of non-specific staining from the gate list statistics generated before for the SSC (SSC/CD19-PE-Cy5, CD14-PE-Cy5, Dead Cell Discriminator) dot plot gated on P1 (Chapter 4, step 2).

Frequencies are determined accordingly, if PBMCs are used.

Example for gate list statistics calculated for whole blood stained with the Anti-BDCA Cocktail and the Control Cocktail, respectively. See Appendix D for typical frequencies in normal healthy blood donors.

Sample ID: Sample
 Total events: 934800
 Gated events: 906068

Gate	Cell type	Events	Gated [%]
P1	Leukocytes	906068	100
P2	-	311034	34.33
P3	MDC1s	1845	0.20
P4	PDCs	1885	0.21
P5	MDC2s	121	0.01

Three-Color
 Four-Color

Sample ID: Control
 Total events: 530865
 Gated events: 518926

Gate	Cell type	Events	Gated [%]
P1	Leukocytes	518926	100.00
P2	-	155876	30.04
P3	Control MDC1s	24	0.00
P4	Control PDCs	26	0.01
P5	Control MDC2s	2	0.00

Three-Color
 Four-Color

6. Determination of the number of DCs of each DC subset per mL of blood

For each DC subset, subtract the percentages of gated events determined for the control sample from the percentages determined for the sample stained with the Anti-BDCA Cocktail. Calculate the absolute numbers of DCs of each DC subset per mL of blood from the corrected frequency and the absolute number of leukocytes per mL of blood as follows:

$$\text{Absolute number of DCs of each DC subset per mL of blood} = \frac{(\% \text{Gated sample} - \% \text{Gated control}) \times \text{Absolute number of leukocytes}}{100}$$

Example: The corrected frequency of MDC1s as determined with the assay was 0.20% (0.20% gated events for the sample minus 0.00% gated events for the control sample). The white blood cell count was 4×10^6 leukocytes per mL of blood. The absolute number of MDC1s per mL of blood was calculated as follows:

$$\text{8000 MDC1s per mL of blood} = \frac{(0.20 - 0.00) \times 4 \times 10^6}{100}$$

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6. Appendix

A: Excitation wavelengths and emission maxima

Excitation wavelengths and emission maxima of the fluorochromes used in the Blood Dendritic Cell Isolation Kit

Fluoro-chrome	Excitation wavelength [nm]	Emission maximum [nm]	Identified cell type
FITC	488	519	PDCs
PE	488	578	MDC1s
DCD	488	625	Dead cells
PE-Cy5	488	670	B cells/monocytes
APC	633/635	660	MDC2s

Three-Color
Four-Color

FITC, PE, PE-Cy5, and the Dead Cell Discriminator (DCD) are excited by a laser emitting light at 488 nm, whereas APC is excited by a laser emitting light at 635 nm. The excitation results in a fluorochrome typical emission spectrum, which is detected, depending on the optical filter configuration of the flow cytometer, in distinct fluorescence channels.

*PE-Cy5 is also excited by laser light at 633/635 nm.

B: Standard flow cytometer configurations

Standard laser and filter configurations of commonly used flow cytometers

Device	MACSQuant Analyzer Miltenyi Biotec GmbH	Epics XL Cytomics FC500	FACScan	FACS Calibur	CyAn
Software	MACSQuantify	Expo	CellQuest		SUMMIT
Standard laser	405 nm 488 nm 635 nm	488 nm	488 nm	488 nm 635 nm	488 nm 635 nm
Fluoro-chrome	Filter	Filter	Filter	Filter	Filter
FITC	FL2	FL1	FL1	FL1	FL1
PE	FL3	FL2	FL2	FL2	FL2
DCD	FL4	FL3	FL3	FL3	FL3
PE-Cy5	FL4	FL4	FL3	FL3	FL4
APC	FL6	–	–	FL4	FL8
Dead Cell Discriminator (DCD)					

Three-Color
Four-Color

C: Surface phenotype of blood dendritic cell subsets

Antigen	PDCs	MDC1s	MDC2s
CD1c (BDCA-1)	-	+	-
CD303 (BDCA-2)	+	-	-
CD141 (BDCA-3)	+	+	++
CD304 (BDCA-4/ Neuropilin-1)	+	-	-
CD4	++	+	+
HLA-DR	+	++	+
CD19	-	-	-
CD3	-	-	-
CD14	-	-/+	-
CD16	-	-	-
CD11c	-	++	+
CD123	++	+	-
CD45RA	+	-	-
CD45RO	-	+	+
CD2	-	+	-
CD32	-	+	-
CD64	-	+	-
FcεRI	-	+	-
CD80	-	-	-
CD83	-	-	-

D: Frequencies and absolute numbers of blood dendritic cell subsets

DC subset	% among leukocytes (range in [%])	Absolute number per mL blood (range in [%])
PDCs	0.19 (0.09–0.37)	1.12×10^4 (4.51×10^3 – 2.04×10^4)
MDC1s	0.27 (0.09–0.42)	1.56×10^4 (6.58×10^3 – 3.28×10^4)
MDC2s	0.02 (0–0.04)	8.60×10^2 (0– 2.16×10^3)

(n=34)

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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