DuraClone IM Phenotyping BASIC Tube, 25 tests, RUO

REF **B53309 – 25 tests**

IFU- B53309-1.0

BECKMAN COULTER

	Specifications of Constituent 1	Specifications of Constituent 2	Specifications of Constituent 3	Specifications of Constituent 4	Specifications of Constituent 5	Specifications of Constituent 6	Specifications of Constituent 7	Specifications of Constituent 8
Specificity	CD16	CD56	CD19	CD14	CD4	CD8	CD3	CD45
Clone	3G8	N901	J3_119	RMO52	13B8.2	B9.11	UCHT-1	J33
Immunogen	Human neutrophils	Human chronic myeloid leukemia cells	SK LY18 Lymphoma Hybridoma	Human monocytes	Human thymocytes	Cytotoxic human T clone HLA A2	T cell line + IL2	Laz 221 cell line
Isotype	IgG1	IgG1	IgG1	IgG2a	IgG1	IgG1	IgG1	IgG1
Species	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse
Source	Ascites fluid or supernatant of in vitro cultured hybridoma cells							
Purification	Affinity chromatography							
Fluorochrome	Fluorescein isothiocyanate (FITC)	R Phycoerythrin (PE)	R Phycoerythrin-Texas Red-X(ECD)	R Phycoerythrin-Cyanine 7 (PC7)	Allophycocyanin(APC)	Alexa Fluor 700 (A700)	Allophycocyanin Alexa Fluor 750 (APC-A750)	Krome Orange
λ Excitation	488 nm	488 nm	488 nm	488 nm	633 nm	633nm	633 nm	405 nm
Emission peak	523 nm	575 nm	613 nm	767 nm	650 nm	720nm	767 nm	528 nm

For Research Use Only.
Not for use in diagnostic procedures.

BACKGROUND

Lymphocytes constitute a part of the human immune system that is referred to as “adaptive” due to their ability to acquire cognition and memory of foreign antigens .[1] B cells (CD45+CD19+) represent the principal part of the humoral branch of adaptive immunity producing antibodies upon terminal differentiation. The cell-mediated adaptive immune response is mainly due to T cells (CD45+CD3+) that comprise a large number of functionally diverse entities with the highest level of discrimination - though not encompassing all T cells, being either CD4+ or CD8+. While CD4+ T cells play a key role in balancing humoral, cell-mediated as well as innate immune responses, e.g. by means of cytokine secretion; CD8+ T cells are mainly responsible for Major Histocompatibility Complex (MHC) - mediated recognition and killing of tumor and virus-infected cells. The CD45+CD3-CD56+ phenotype identifies Natural Killer (NK) cells that complement the specificity of T cell receptors and immunoglobulins by the recognition of the “missing self”, i.e. the absence of host-matched MHC molecules presenting processed antigens on the membrane surface. Being a part of the innate human immune system, monocytes (CD45+CD19+) play a key role in bacterial defense. Expression of the Lipopolysaccharide (LPS, endotoxin) receptor CD14 is dense on the majority of monocytes bearing a classical phenotype.[2]

APPLICATION

The DuraClone IM panels are used to identify cell subpopulations in human whole blood samples by flow cytometry.

The IM Phenotyping BASIC Tube is an 8-color, 8-monoclonal antibody reagent that allows the identification of common extracellular markers of different subpopulations of lymphocytes, present in whole blood specimens.

This reagent is intended to be used on a flow cytometer with three lasers:

- A 488 nm laser capable of detecting light scatter (forward and side) and a fluorescence emission in the following ranges: 504-545 nm, 560 – 600 nm, 605 – 635 nm, 680 –710 nm and >755nm.
- A 638 nm laser capable of detecting light emission in the following ranges: 650-670 nm, 715-735nm and > 755 nm.
- A 405 nm laser capable of detecting the fluorescence emission in the following ranges: 430-470 nm and 530-570nm.

PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by lymphocytes. Specific staining of the leukocytes is performed by incubating the sample with IM Phenotyping BASIC Tube. The erythrocytes are then removed by lysis and the leukocytes, which are unaffected by this process of lysis are acquired and analyzed by flow cytometry. The flow cytometer measures light diffusion and the fluorescence of cells; it enables the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light(Side Scatter or SS) and the diffusion of narrow angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage. The fluorescence of the delimited cells is analyzed in order to distinguish the positively stained events from the unstained events. The gating strategy allows detection of circulating lymphocyte subpopulations. The results are expressed as a percentage of positive events.

KIT BOX CONTENTS

DuraClone IM Phenotyping BASIC Tube, 25 tests, RUO contains the following:

- 25 tests of the DuraClone IM Phenotyping BASIC Tube (i.e. a single tube is a single test)
- 3Compensation Kits, each kit containing eight tubes, each of a single color; i.e.
  - CD4-FITC
  - CD4-PE
  - CD19-ECD
  - CD14-PC7
  - CD4-APC
  - CD8-A700
  - CD3-APC-A750
  - CD8-Krome Orange

STATEMENT OF WARNINGS

1. Do not use the reagent or compensation tubes beyond the expiry date.
2. Do not store the tubes in the refrigerator; do not freeze/thaw the tubes.
3. All blood samples must be considered as potentially infectious and must be handled with care (protective gloves, gowns and goggles must be used while handling blood samples).
4. Tubes containing blood and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.
5. Minimize the exposure of light to the tubes, especially during incubation of sample stained with fluorescent antibodies or during lysis and after processing of sample, before use.
6. A calibrated pipette should be used for the addition of blood samples and the pipette should be operated according to the manufacturer's instructions.

STORAGE CONDITIONS

Store the reagent tubes and compensation kit tubes between 20 and 30°C, in a dry place and protect it from the direct exposure to light and moisture. Refer to the kit label for the date of expiry of the reagent.

EVIDENCE OF DETERIORATION

Any damage to the panel tube may indicate product deterioration and the product should not be used. Please contact your local distributor or you can contact Beckman Coulter at the following email address: duraclone-support@beckman.com

INSTRUMENT REQUIREMENTS

This reagent is designed to be used on a flow cytometer capable of detecting forward and side scatter, and compatible with the emission spectra of the fluorochromes used in the reagent. This reagent is compatible with *Navios.

SPECIMEN COLLECTION

The venous blood sample should be collected in a blood collection tube containing anticoagulant. Follow the collection tube manufacturer’s guidelines for the minimum volume of blood to be collected. The sample must be stored between 18°C and 26°C.

MATERIAL REQUIRED BUT NOT SUPPLIED

- Blood collection tube containing anticoagulant
- Calibrated pipettes
- Vortex mixer
- Sheath fluid
- Flow cytometer calibration beads
- Flow-Check Pro Fluorospheres (REF. A69183) (For Navios alignment verification)
- Flow-Set Pro Fluorospheres (REF. A69184) (For Navios standardization)
- VersaLyse Solution (REF. A09777)
- IOTest 3 Fixative Solution (REF. 8546859)
- Flow cytometer

PROCEDURE

SAMPLE PREPARATION

1. Add 100µL of fresh whole blood to the dried reagent tube, vortex at high speed for 6-8 seconds and incubate the tube for 15 minutes, protected from the direct exposure to light between 20 and 30°C.
2. Add 2 mL of VersaLyse, vortex the tube at high speed for 1-3 seconds and incubate the tube for 15 minutes

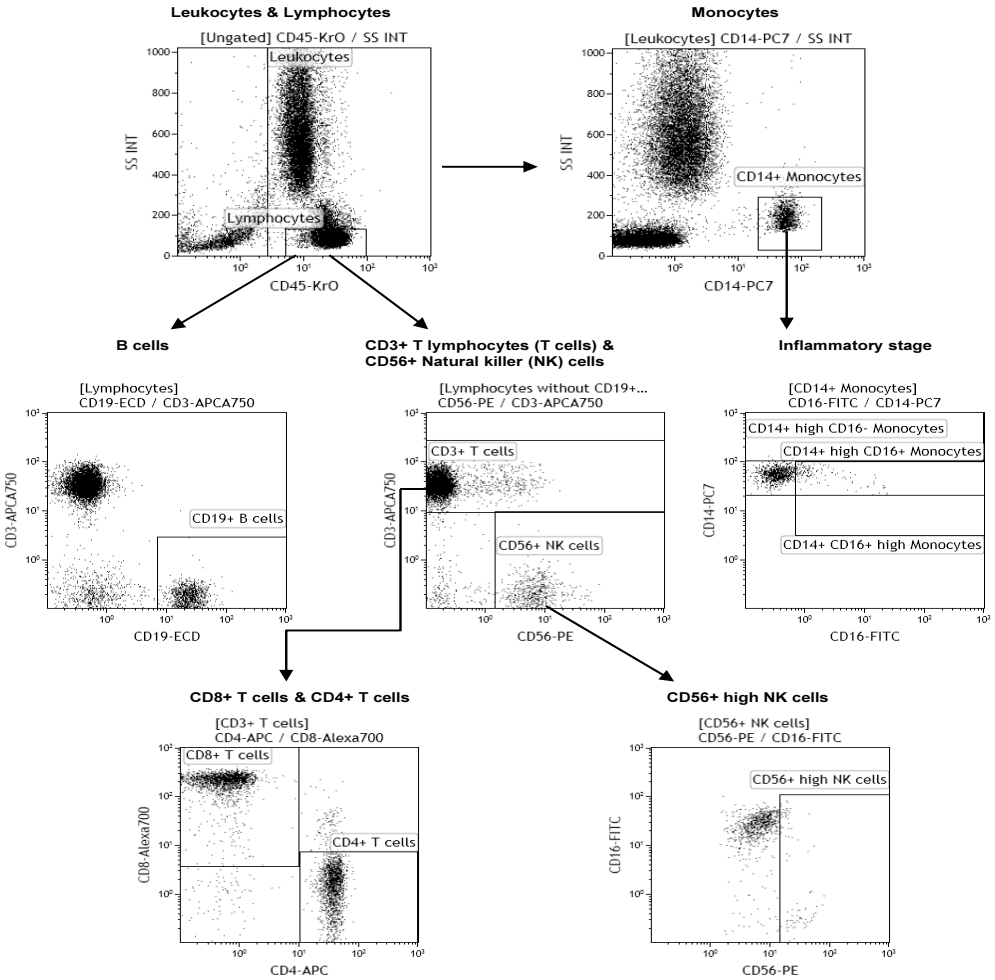
protected from the direct exposure to light, between 20 and 30°C.

3. Centrifuge the tube at 200 x g for 5 minutes; aspirate the supernatant, gently tap the cell pellet.
4. Perform a wash step by re-suspending the cell pellet in 3mL 1X PBS and centrifuging the tube at 200 x g for 5 minutes; aspirate the supernatant, gently tap the cell pellet and re-suspend the cell pellet in 500 µL of 1X PBS containing 0.1% IOTest 3 Fixative Solution. The sample is now ready for acquisition.

COMPENSATION SETUP

1. Stain all the eight single color tubes from a single pouch of the Compensation Kit provided in the IM DuraClone Phenotyping BASIC Tube, 25 tests, RUO with venous blood by following steps 1-4 in the sample preparation procedure.
2. For sample acquisition on Navios: The AutoSetup Scheduler on the Navios groups the selected applications for efficient set up in sampling from common compensation samples when scheduling multiple applications and provides the carousel load report to facilitate setting up and loading samples for daily QC. For setting up compensation using AutoSetup Scheduler, refer to the Application Note "Compensation Setup for High Content DuraClone reagents", downloadable from the Beckman Coulter website: www.duraclone.com
3. For all other flow cytometer users, please follow standard procedures and instrument manufacturer instructions for compensation setup.

SAMPLE ANALYSIS (Recommended)



1. Create an appropriate analysis protocol to define the population gates and the series of dual parameter plots for analysis of the reagent specificities.
2. Set the discriminator on the FS parameter such that the lymphocytes are not excluded from the acquisition.
3. Create a CD45-KrOrange (Krome Orange) vs. SSC dot plot and create a region to encompass the CD45+ leukocytes
4. Create three plots as follows:
   a. Create a CD14- PC7 vs. SSC dot plot and apply the CD45+leukocyte gate onto this plot and draw a region to encompass the CD14+ cells. These cells are the monocytes.
   b. Create a CD19- ECD vs. D3-APC-A750 dot pot and draw a region to encompass the CD19+ cells. These cells are the B lymphocytes (i.e. B cells).
   c. Create a CD56-PE vs.CD3-APC-A750 dot plot. Create a Boolean gate "Lymphocytes AND (NOT CD19+)" and apply this gate i.e. lymphocytes without the CD19+ B cells) to the plot. Draw a region to encompass the CD56+ and CD3+ cell populations. TheCD56+ cells are the Natural Killer (NK) cells and the CD3+ cells are the CD3 + T lymphocytes (T cells).
5. Create a CD56-PE vs. CD16-FITC dot plot. Apply the gate from the CD56-PE vs.CD3-APC-A750 dot plot. Draw a region to encompass the CD56+ high NK cells.
6. Create a CD4-APC vs. CD8-A700 (i.e. Alexa Fluor 700) dot plot. Apply the gate CD3+ Tcells gate from the CD56-PE vs.CD3-AA750 dot plot. Draw a region to encompass the CD4+ T cells and another region to encompass the CD8+ T cells.
7. Create CD16-FITC vs. CD14-PC7 dot plot. Apply the CD14+ gate onto this plot. Draw regions to encompass the following cell populations:-
   a. The CD 14+ high CD16- monocytes
   b. The CD14 + high CD16+ monocytes
   c. The CD14+ CD16 high monocytes
8. Record the % recruitment and the mean fluorescence intensity (MFI) of all the gated cell populations.

REFERENCES

1. Paul W.E. (ed.) (2003). Fundamental Immunology (4th ed.).Philadelphia: Lippincott-Raven.
2. Ziegler-Heitbrock L, Hofer TP. Toward a refined definition of monocyte subsets. Front Immunol. 2013 Feb 4; 4:23.

PRODUCT AVAILABILITY

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TRADEMARKS

For additional information, or if a damaged product is received, email Beckman Coulter Customer Service at duraclone-support@beckman.comor contact your local Beckman Coulter Representative.

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- Initial Release