DuraClone IM B cells Tube, 25 tests, RUO

REF **B53318 – 25 tests**

IFU- B53318-1.0

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	IgD	CD21	CD19	CD27	CD24	CD38	IgM	CD45
Clone	IA6-2	BL13	J3-119	1A4CD27	ALB9	LS198-4-3	SA-DA4	J33
Immunogen	Human IgD	Human B-CLL lymphocytes	SK LY18 Lymphoma Hybridoma	PHA stimulated human T cells	Bone marrow malignant cells	Human T cell line HUT 78	Human Ig heavy-chain from myeloma cells	Laz 221 cell line
Isotype	IgG2a	IgG1	IgG1	IgG1	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)	Allophycocyanin-Alexa Fluor 750 (APC-A750)	Pacific Blue	Krome Orange
λ Excitation	488 nm	488 nm	488 nm	488 nm	633 nm	633 nm	405 nm	405 nm
Emission peak	523 nm	575 nm	613 nm	767 nm	650 nm	720 nm	455 nm	528 nm

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

BACKGROUND

The major function of B cells (CD45+CD19+) in the human immune system is to recognize and memorize immunogenic structures and to terminally differentiate into plasma cells that produce antibodies against these recognized or memorized antigens. Late maturation stages of B cells are confined to peripheral compartments and can be described based on a previously published classification.[1] The passage from bone marrow into peripheral compartments is associated with surface expression of immunoglobulin of the isotype classes M and D (IgM, IgD). Among IgM+ B cells high expression of CD24 along with absence of CD27 identifies transitional B cells, the earliest stage of maturation in peripheral compartments. Acquisition of CD27 expression identifies transition from a naïve to a marginal zone phenotype, further acquisition of low CD38 expression reveals memory identity (non-isotype -class-switched). Loss of IgM and IgD surface expression is indicative of immunoglobulin isotype class switching giving rise to CD27+CD38- class-switched memory cells or CD27+CD38++ plasmablasts.

APPLICATION

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

The DuraClone IM B cells Tube is an 8-color, 8-monoclonal antibody reagent that allows the identification of B lymphocyte subpopulations present in whole blood samples. 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 B lymphocytes. Specific staining of the leukocytes is performed by incubating the sample with IM B cells Panel. The erythrocytes are then removed by lysis and the leukocytes, which are unaffected by this process, are acquired and analyzed by flow cytometry. The flowcytometer 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 ones. The gating strategy allows detection of circulating B cell subpopulations. The results are expressed as a percentage of positive events.

KIT BOX CONTENTS

The DuraClone IM B cells Tube, 25 tests, RUO contains the following:-

- 25 tests of the DuraClone IM Basic Tube (i.e. a single tube is a single test).
- 3 compensation kits; each kit containing eight tubes, each containing a single color i.e.:
  - CD4-FITC
  - CD4-PE
  - CD19-ECD
  - CD27-PC7
  - CD4-APC
  - CD38-APC-A750
  - CD4-Pacific Blue
  - 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 tubes of the DuraClone IM B cells Tube, 25 tests, RUO as well as the tubes from the compensation kit between 20°C and 30°C, in a dry place and protect them 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 reagent 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

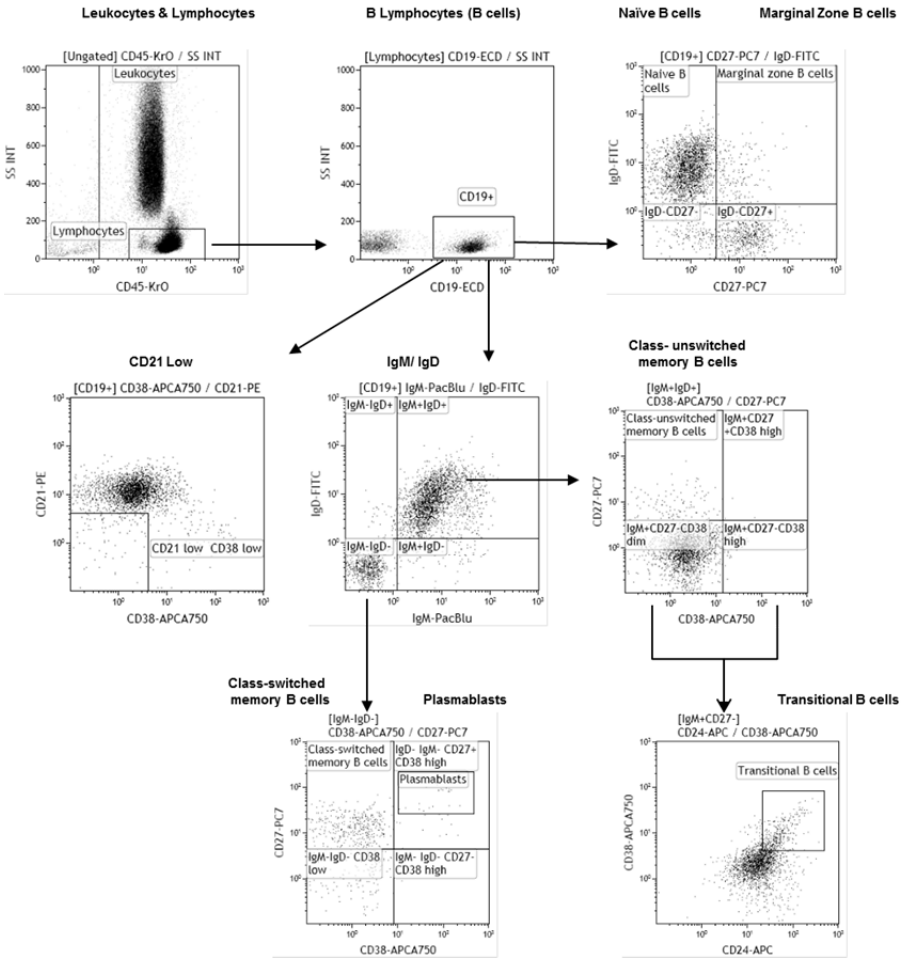
1. Add 10mL 1X PBS to 300 µL of fresh whole blood, to a 15 mL conical tube and centrifuge the tube at 300 x g for 10 minutes; aspirate the supernatant and re-suspend the pellet in 10 mL of 1X PBS. Centrifuge the tube again at 300 x g for 5 minutes aspirate the supernatant and re-suspend the pellet in 300 µL of 1X PBS.
2. Add 100 µL of washed whole blood to 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.
3. Add 2 mL of VersaLyse Solution, 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.
4. Centrifuge the tube at 200 x g for 5 minutes; aspirate the supernatant, gently tap the cell pellet.

5. 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 500µL of 1X PBS containing 0.1% IO Test 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 B cells Tube, 25 tests, RUO with 100 µL of fresh whole blood each, incubate all the eight tubes for 15 minutes, protected from the direct exposure to light, between 20 and 30°C and then follow steps 3-5 in the Sample Preparation procedure for preparing the eight compensation tubes.
2. For sample acquisition on Navios: The AutoSetup Scheduler on the Navios flow cytometer 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.

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 a CD19- ECD vs. SSC dot pot and create a region to encompass the CD19+ cells. The CD19+ cells are the B lymphocytes (i.e. B cells).
5. Create three dot plots and apply the CD19+ gate on all the three plots:
   a. CD27-PC7 vs. IgD-FITC: Draw a quadrant to gate the IgD+ CD27- cell population; this is the naïve B cell population. Gate the IgD+ CD27+ population; these are the marginal zone B cells.
   b. CD38 -AA750 vs. CD21-PE. : Draw a quadrant to delineate the CD21 low CD38 low cell population.
   c. IgM-PacBlu (i.e. Pacific Blue) vs. IgD-FITC: Draw a quadrant to delineate the IgD-IgM-, IgD+IgM- , IgD+IgM+, IgD-IgM+ cell populations.
6. Create a CD38 AA750 vs. CD27-PC7 dot plot and apply the IgD-IgM- gate onto the plot. Draw a quadrant to delineate the following:
   a. IgD-IgM- CD38-CD27+population: These are the class-switched memory B cells.
   b. IgD-IgM- CD38-low CD27- population.
   c. IgD-IgM- CD38+ high CD27- population.
   d. IgD-IgM-CD38+ high CD27+ population: These are the plasmablasts.
7. Create the following dot plots and apply the IgD+IgM+ gate onto them:
   a. CD38-APC-A750 vs. CD27-PC7: Draw a quadrant to delineate the following:
      1) IgM+CD27-CD38 dim population
      2) IgM+CD27+CD38- population: These are the class-unswitched memory B cells.
      3) IgM+CD27+ CD38+high population
      4) IgM+ CD27-CD38- population.
   b. CD24-APC vs. CD38-APC-A750: Draw a region to encompass the transitional B cells by applying the Boolean gate IgM-CD27-CD38 dim OR IgM-CD27-CD38 high.

REFERENCES

1. The EUROclass trial: defining subgroups in common variable immunodeficiency. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, Vlkova M, Hernandez M, Detkova D, Bos PR, Poerksen G, von Bernuth H, Baumann U, Goldacker S, Gutenberger S, Schlesier M, Bergeron-van der Cruyssen F, Le Garff M, Debré P, Jacobs R, Jones J, Bateman E, Litzman J, van Hagen PM, Plebani A, Schmidt RE, Thon V, Quinti I, Espanol T, Webster AD, Chapel H, Vihinen M, Oksenhendler E, Peter HH, Warnatz K. Blood. 2008 Jan 1; 111(1):77-85.

PRODUCT AVAILABILITY

DuraClone IM B cells Tube, 25 tests, RUO
REF B53318

TRADEMARKS

Beckman Coulter, the stylized logo, Navios and VersaLyse Solution are trademarks of Beckman Coulter, Inc., and are registered in the USPTO. DuraClone, Flow-Check, Flow-Set and Krome Orange are trademarks of Beckman Coulter, Inc.

*Navios is CE marked for 10-color in vitro diagnostic (IVD) use. In the U.S.A., Navios is intended for use as an IVD device for immunophenotyping with Navios tetra software and CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/ CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 reagents. All other uses are for research use only (RUO).

IOTest is a trademark of Immunotech S.A. and is registered in the USPTO.

Alexa Fluor and Pacific Blue are registered trademarks of Molecular Probes, Inc.

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.

Beckman Coulter India Pvt. Ltd.
50-B, II Phase, Peenya Industrial Area
Peenya, Bangalore 560058, India

Printed in India

© 2014 Beckman Coulter, Inc.
All Rights Reserved.

Revision 1.0, Spetember 2014

- Initial Release