

## DuraClone IF Basophil Activation Tube

Specificity	CD45	CD3	CD294 (CRTH2)	CD203c	CD63
Clone	J33	UCHT1	BM16	97A6	CLB-Gran/12
Hybridoma	NS1 x balb/c	NS1 x balb/c	SP2/0 x rat	SP2/0 x balb/c	SP2/0 x (balb/c x AJ)
Immunogen	Laz 221 cell line	T cell line + IL2	CRTH2 transfected cell line (TART/B9-12.10)	UT-7 megakaryoblast cell line	Human cytochrome B enriched cells
Immunoglobulin	IgG1	IgG1	IgG2a	IgG1	IgG1
Species	Mouse	Mouse	Rat	Mouse	Mouse
Purification	Affinity Chromatography				
Fluorochrome	Krome Orange	R Phycoerythrin - Cyanine 7 (PC7)	Alexa Fluor 647	R Phycoerythrin (PE)	Pacific Blue
Molar Ratio	Krome Orange / protein : 10,40 - 14,10	PC7 / protein : 0,5 - 1,5	Alexa Fluor 647 / protein : 1,93 - 2,58	PE / protein : 0,5 - 1,5	Pacific Blue / protein : 4,83 - 6,59
$\lambda$ excitation	405 nm	488 nm	633/638 nm	488 nm	405 nm
Emission Peak	528 nm	770 nm	665 nm	575 nm	455 nm
Buffer	PBS pH 7.2 plus 2 mg/ mL BSA and 0.1% NaN3				

**REF** C23406 - 25 tests

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

### INTENDED USE

DuraClone IF Basophil Activation Tube, 25 Tests, RUO is intended to study the in vitro IgE-dependent responses to allergen in sensitized individuals by flow cytometry analysis of basophils. This kit consists of 25 tubes of a dried optimized five-color combination of fluorescent monoclonal antibodies as well as 5 tubes of a positive control containing the same dried five color cocktail along to an anti-IgE also dried. The reagents of this kit are designed to characterize the activation status of basophils. For this purpose, a 5-color combination is used. It is a mixture of 4 fluorescent murine monoclonal antibodies (CD203c-PE, CD3-PC7, CD63-PBE, CD45-KrO) and 1 fluorescent rat monoclonal antibody (CRTH2-AF647).

### SPECIFICITY

Basophils play a key role in immediate hypersensitivity as primary effector cells. These cells express the high affinity receptor for IgE (Fc $\epsilon$ R1). Allergens induce basophils activation by cross-linking surface IgE leading to release of mediators and expression of activation markers on the basophils surface such as CD63 and CD203c.

The analysis of level of expression of CD63 and CD203c antigen allows the characterization of resting and activated basophils in an IgE-dependent response to allergens in sensitized individuals (1,2). Interestingly, CD203c and CD63 expression do not always correlate as they seem to implicate 2 different pathways of basophils activation where CD63 will reflect anaphylactic degranulation and CD203c piecemeal degranulation (3).

CD203c-PE / CD3-PC7 / CRTH2-AF647 / CD63-PB / CD45 KrO REAGENT

### CRTH2

CRTH2 is a seven-transmembrane molecule known as the Chemoattractant Receptor-homologous molecule. Among normal whole blood leucocytes, CRTH2 is highly expressed on basophils and eosinophils, as well as on Th2 and Tc2 cells that are known to be responsive for humoral immune responses and allergy (4,5).

### CD3

The CD3 antigen is expressed only on mature T lymphocytes and on a sub-population of thymocytes (6). In peripheral blood, approximately 67 to 76% of lymphocytes are CD3<sub>pos</sub>; this percentage is lower in young children and varies according to age (7).

### CD203c

Basophils and mast cells are hematopoietic effector cells that highly express the high affinity IgE receptor (Fc $\epsilon$ R1). MA b 97A6 recognizes a surface antigen expressed on human peripheral blood basophils, but not on other blood cells (8). It also reacts with mature mast cells, and with CD34<sub>pos</sub> bone marrow progenitors of basophils and mast

cells. Moreover the 97A6 antigen is up-regulated after activation of basophils by anti-IgE antibodies and various allergens (9).

### CD63

CD63 was first described in granules of resting platelets and on the surface membrane on activated platelets (2) Its surface expression is associated to lysosomal secretion: CD63, is strongly expressed at the surface of basophils after activation (10,11) and it's expression have been inversely correlated with Histamine level in basophils (12). The CLBGran/12 monoclonal antibody (mAb) reacts with most peripheral blood cells including activated platelets, lymphoid, myeloid and endothelial cells, except with red blood cells and resting T cells.

### CD45

CD45 epitope is present on the surface of all human leucocytes; lymphocytes, eosinophils monocytes, basophils and neutrophils, by order of decreasing level of expression. CD45 is a major component of the lymphocyte membrane. It is absent from erythrocytes and platelets (13).

## PRINCIPLE

The cell population of interest is stained with monoclonal antibodies in the presence of the allergens and respective positive or negative controls. Erythrocytes are then lysed prior to flow cytometry analysis. Once excluding T lymphocytes (CD3 positive cells) and granulocytes, basophils are analyzed using CRTH2, CD203c and CD63 expression. Non-activated and resting basophils are identified as (lymphocytes+monocytes) CD45<sub>pos</sub> CD3<sub>neg</sub> CRTH2<sub>pos</sub> CD203c<sub>dim</sub> CD63<sub>neg</sub>, whereas in vitro activated basophils are identified as (lymphocytes+monocytes) CD45<sub>pos</sub> CD3<sub>neg</sub> CRTH2<sub>pos</sub> CD203c<sub>bright</sub> CD63<sub>pos</sub>.

## WARNING AND PRECAUTIONS

1. Specimens, samples and all material coming in contact with them should be considered potentially infectious and disposed of with proper precautions.
2. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
3. Do not use antibody beyond the expiration date on the label.
4. Do not expose reagents to strong light during storage or incubation.
5. Use good laboratory practices when handling this reagent.
6. Any change in the physical appearance of the reagents may indicate deterioration and the reagent should not be used.
7. Seal the zip lock of pouch containing reagent tubes after removing the desired number of tests.
8. Reagents tubes must be stored within the sealed pouch containing dessicant packs prevent the tubes from being exposed to moisture.

## GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS	Safety Data Sheet is available at <a href="http://techdocs.beckmancoulter.com">techdocs.beckmancoulter.com</a>
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## STORAGE AND HANDLING CONDITIONS AND STABILITY

Store the tubes of the kit between 18°C and 25°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](mailto:duraclone-support@beckman.com)

## MATERIALS REQUIRED, BUT NOT PROVIDED

Deionized water.

PBS buffer (e.g. Beckman Coulter PN 6602489).

Allergens.

Activation Solution (e.g Beckman Coulter Ref C23407)

Lysis solution (e.g Optilyse C, Beckman Coulter Ref A11894)

37°C water bath.

Flow Cytometer.

## **PROCEDURE**

### **IMPORTANT:**

- The following procedure is optimized for whole blood specimen collected with EDTA as anticoagulant.
- Fresh heparinized whole blood may be used following this procedure by replacing in step 1 and step 2 the Activation Solution by PBS1X.
- Bring Activation Solution to room temperature (18 – 25°C) prior to experiment.
- Each laboratory shall establish the right range of allergens dilutions to be used for the experiment.
- CRTH2 expression level might vary among donors. Using the wash procedure or increasing the incubation time to 30 min in the no wash procedure will improve the separation between CRTH2<sub>pos</sub> and CRTH2<sub>neg</sub> cells.

### **No wash procedure**

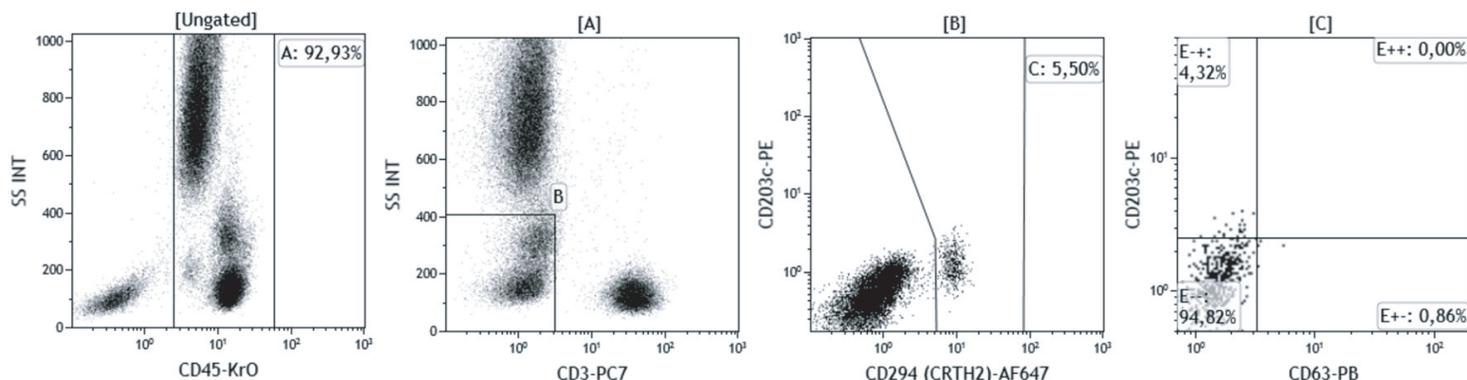
1. Prepare allergen dilutions in activation solution
2. Add 50 µL of Activation Solution (with or without allergen) into each tube
3. Vortex at high speed for 6 to 8 seconds
4. Add 50µl of blood
5. Gently vortex each tube for 1 to 2 seconds and incubate for 15 minutes at 37°C on water bath, protected from light.
6. Remove tubes from water bath and add 250µl of OptiLyse C under fume hood and vortex immediately for 1 to 2 seconds (proceed tube by tube to avoid incomplete lysis).
7. Incubate at room temperature (18 – 25°C) for 10 minutes, protected from light.
8. Add 250µl of PBS and vortex immediately for 1 to 2 seconds
9. Incubate at room temperature (18 – 25°C) for 10 minutes, protected from light.
10. Acquire on the flow cytometer.

### **For the procedure with an optional wash**

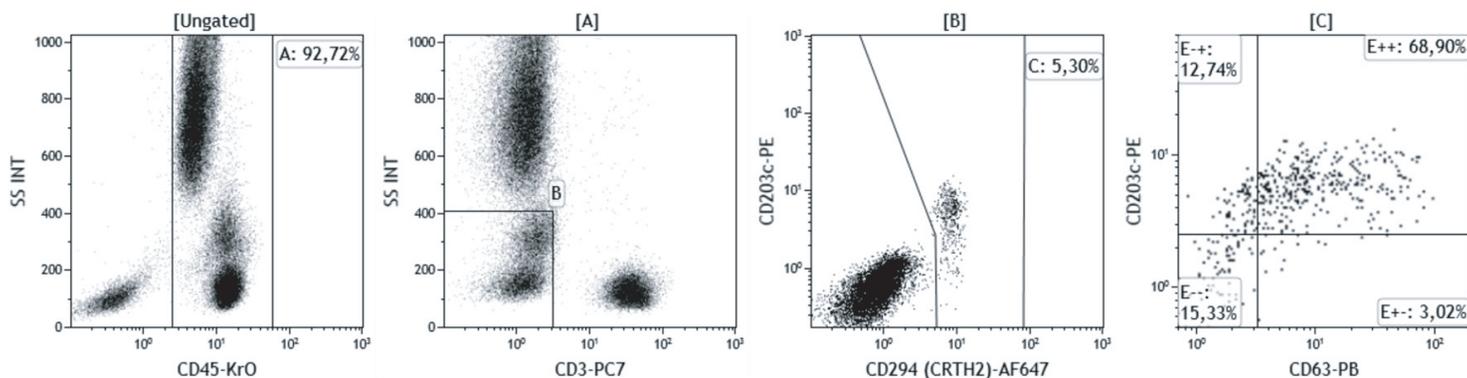
1. Proceed normally from step 1 to 9, and add 3ml of PBS.
2. Centrifuge for 5 minutes at 300 x g at room temperature.
3. Remove the supernatant by aspiration.
4. Resuspend the cell pellet in 0.5ml of PBS plus 0.1% of formaldehyde.
5. Acquire on the flow cytometer.

## EXAMPLE DATA

### Test tube Without Allergen



### Positive Control tube



## LIMITATIONS

Flow cytometry may produce false results if the cytometer has not been aligned perfectly.

The conjugated antibody of this reagent is calibrated so as to offer the best specific signal / non-specific signal ratio.

Accurate and reproducible results will be obtained as long as the procedures used are in accordance with the technical insert leaflet and compatible with good laboratory practices.

Verify the preparations using the naked eye to assess the efficacy of lysis. If they are cloudy or if the light diffraction histograms are unusual, it may be that lysis is incomplete.

The erythroblasts may be incompletely lysed and appear on a light diffraction histogram in the same location as the leucocytes.

Test Tubes and Positive control Tubes contain BSA, positivity might be observe on blood from donor presenting reactivity to BSA (14).

As described in literature, Heterogeneity of basophils activation levels between donors can be observed (15).

Non-responders donors with basophils that do not respond to basophils activation have been described (16).

Blood should be used shortly after drawing as it might affect basophils activation detection (17).

## TRADEMARKS

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## ADDITIONAL INFORMATION

For additional information, or if damaged product is received, call Beckman Coulter Customer Service at 800-526-7694 (USA or Canada) or contact your local Beckman Coulter Representative.

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IMMUNOTECH S.A.S. a Beckman Coulter Company, 130, avenue de Lattre de Tassigny,  
BP 177, 13276 Marseille cedex 9, France, 33-491 172 727