IOTest CD294 (CRTH2)-PE

PN A07413 – 100 tests – Liquid – 20 µL/test – Clone BM16

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

SPECIFICITY

The cytokines produced by Th1 and Th2 lymphocyte subsets determine different pathways of the immune response. Activated $CD4^{pos}$ T lymphocytes of the Th1 profile secrete IL-2, IFNγ (interferon γ), and TNFβ (Tumor Necrosis Factor β). Th1 profile of cytokines secretion is reported to be involved in cellular immunity, delayed type hypersensitivity reactions (DHT) and activation of cytotoxic and inflammatory functions (1). Activated $CD4^{pos}$ T lymphocytes of the Th2 profile produce essentially IL-4, IL-5 and IL-10: they are known to be responsive for humoral immune responses and allergy. Th1 and Th2 pathways each enhance the development of cells pertaining to the same subset while suppressing the expansion and/or effector functions of the other subset (2 – 5).

The Th1- and Th2-cytokine profiles are not specifically produced by Th lymphocytes ($CD4^{pos}$), but also by Tc lymphocytes ($CD8^{pos}$), allowing for a generalized nomenclature (6, 7): Th1- and Th2-like cytokine profiles may be termed Type 1 and Type 2 responses (1, 2, 4, 6).

CD294 also known as CRTH2, is a seven-transmembrane molecule known as the Chemoattractant Receptor-homologous molecule expressed on Th2 cells. The deduced amino acid sequence of CD294 suggests two potential N-glycosylation sites at the first extracellular domain and a unique long cytoplasmic tail including four consensus sites for protein kinase C phosphorylation. Two G protein-coupled receptors, Prostaglandin D receptor (DP), and CD294 have been identified as receptors for prostaglandin D2 (PGD2), but they differ in their signaling pathways (8, 9). PGD2 is the major metabolite of arachidonic acid produced by allergen-activated mast cells and has been implicated in various allergic diseases as a proinflammatory lipid mediator (8).

CD294 molecule is preferentially expressed on human T-helper (Th) 2 and T cytotoxic (Tc) 2 cells but not on Th1 and Tc1 cells (10). CD294 is the most reliable surface marker selectively associated with circulating T (Th and Tc) cells able to produce IL-4 (as well as IL-5 and IL-13) and but IFNγ (10, 11). Among normal whole blood leucocytes, CD294 is highly expressed on basophils and eosinophils, as well as on Th2 and Tc2 cells. The expression of CD294 in Peripheral Blood Mononuclear Cells (PBMCs) is highly restricted to an activated state of Type 2 cells: i.e. CD294 is not expressed by $CD25^{neg}$ T cells (12). CRTH2 may be expressed (weakly) on some monocytes and/or dendritic cells ($CD14^{dim}$, $CD16^{pos}$, $HLA\text{-}DR^{pos}$, $CD33^{pos}$) (8, 13).

CD294 may act in mediating the recruitment and/or activation of basophils, eosinophils and Th2 cells at sites containing mast cells activated by invading allergens (14). CD294, but not DP induces migration of Th2 cells, eosinophils and basophils in response to PGD2 (9).

The BM16 monoclonal antibody precipitates a 55 to 70 kDa protein from cells lysates of CD294-transfected Jurkatt and from established Th2 clone, (e.g. clone 6L21) corresponding to PGD2 receptor (12).

The BM16 monoclonal antibody has been assigned to the CD294 cluster of differentiation during the 8^{th} HLDA, Workshop on Human Leukocyte Differentiation Antigens, held in Adelaide, Australia, in 2004 (15)

REAGENT

IOTest CD294 (CRTH2)-PE
Conjugated antibody
PN A07413 - 100 tests - Liquid - 20 µL/test

Clone	BM16
Isotype	IgG2a, Rat
Immunogen	CD294 transfected cell line (TART/B19-12.10)
Hybridoma	SP2/0 x rat
Source	Ascites fluid or supernatant of in vitro cultured hybridoma cells.
Purification	Ion exchange or affinity chromatography
Conjugation	R Phycoerythrin (PE)
Molar Ratio	PE / Ig : 0.5 - 1.5
Fluorescence	Excites at 488 nm Emits at 575 nm

REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin.

APPLICATION

Flow cytometry.

STATEMENTS OF WARNING

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be considered potentially infectious and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Use good laboratory practices when handling this reagent.

STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze.

REAGENT PREPARATION

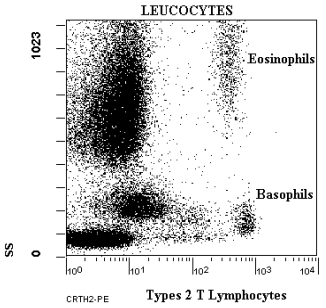
No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

PROCEDURE

This reagent is designed for Flow Cytometry.
Assay volume: 20 µL per 5 x 10^5 cells in one test, or per 100 µL whole blood.
A wash is required to yield optimal results.

EXAMPLE DATA

The histogram below is a biparametric representation (Side Scatter *versus* Fluorescence Intensity) of a normal human whole blood sample. Staining is performed with IOTest CD294-PE Conjugated Antibody on leucocytes. Erythrocytes lysis is performed with VersaLyse Lysing Solution (See catalog for PN), and fixation with IOTest 3 Fixative Solution (See catalog for PN).



Acquisition is performed with a COULTER EPICS XL flow cytometer equipped with System II software.
Analysis is performed with EXPO 32 software.

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