IOTest Anti-Human IgD-APC

PN B30651 – 50 tests – Liquid – 10 µL/test – Clone IA6-2

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

SPECIFICITY

The anti-Human IgD IA6-2 monoclonal antibody (mAb) binds specifically to the heavy chain of human IgD (1).

IgD, a member of the immunoglobulin (Ig) family, is expressed in naïve B cells. It exists in a transmembrane and a soluble form (2).

IgD is, together with IgM, the first antibody isotypes expressed during B cell ontogeny. Bone marrow B cell precursors acquire surface IgM after assembling heavy (H) and light (L) chain variable region exons from prototypic variable (V), diversity (D) and joining (J) gene segments through an antigen-independent process. After leaving the bone marrow to colonize secondary lymphoid organs, B cells acquire surface IgD of the same specificity as surface IgM through alternative splicing of a pre-messenger RNA comprising V(D)J and both heavy chain constant µ (Cµ) and Cδ exons. Class switch recombination (CSR) and somatic hypermutation (SHM) require activation-Induced Cytidine Deaminase (AID) (3). The significance of dual IgM and IgD expression remains unclear, because either isotype largely compensates for the loss of the other (4, 5). IgM+IgD+CD27+ B cells represent 10 to 30% of total B cells in normal individuals.

Human B cells release IgD antibodies in the blood as well as respiratory, salivary, lacrimal and mammary secretions (4). Circulating IgD bound to basophils through a calcium-mobilizing receptor that activated antimicrobial, opsonizing, pro-inflammatory and B cell-stimulating programs upon cross-linking. Both IgD class-switched B cells and IgD armed basophils were dysregulated in patients with autoinflammatory syndromes and periodic fever, indicating that IgD plays a role at the interface between immunity and inflammation (2, 4). This evolutionarily conserved immune surveillance system would not only monitor systemic invasion by airborne pathogens at the upper respiratory tract, but also regulate B cell homeostasis, antibody production and inflammation (6).

REAGENT

IOTest Anti-Human IgD-APC
Conjugated Antibody
PN B30651 – Liquid - 50 tests - 10 µL/test

Clone	IA6-2
Isotype	IgG2a, Mouse
Immunogen	Human IgD
Hybridoma	Murine hybridoma IA6-2
Source	Purified
Purification	Affinity chromatography
Conjugation	Allophycocyanin (APC)
Molar Ratio	APC / Ig : 0.5 - 1.5
Fluorescence	Excites at 633/638 nm Emits at 660 nm

REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin. Concentration: See lot specific Certificate of Analysis at www.beckmancoulter.com.

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.
8. Any change in the physical appearance of the reagents may indicate deterioration and the reagent should not be used.

STORAGE AND HANDLING CONDITIONS AND STABILITY

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

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

PROCEDURE

SPECIMEN PREPARATION

Both whole blood and bone marrow specimens are pre-washed prior to staining to avoid plasma/serum protein interferences. Based on the individual laboratory workflow, specimens can be washed using a bulk or single tube procedure.

NOTE: Single cell suspensions prepared from lymphoid tissues may not require washing prior to staining if the specimen was washed during the disaggregation process. If washing steps were not performed for removal of residual soluble proteins, or if the cells were resuspended into a buffer containing human serum or serum proteins, then pre-washing is necessary. Follow your laboratory procedure for washing.

CAUTION: Failure to follow the washing instructions (volumes and wash cycles) may cause erroneous results.

A. Bulk Wash Procedure

1. Obtain WBC count of the sample.
2. Add 1.0 mL whole blood or bone marrow specimen to a 15 mL conical centrifuge tube.
3. Add 9.0 mL of the PBS/ 2% FCS wash buffer. Mix by gentle inversion.
4. Centrifuge at 150 x g for 10 minutes.
5. Aspirate and discard supernatant.
6. Repeat steps 3 to 5 two additional times.
7. Resuspend the washed pellet in either PBS/ 2% FCS or PBS/ 50% mouse serum with an appropriate volume to obtain a WBC count between 2-20 x 10^3 cells/µL.
8. Proceed to Staining Procedure.

B. Single Tube Wash Procedure

1. Obtain WBC count of the sample.
   a. If the WBC count is above 20 x 10^3 cells/µL, dilute sample appropriately with the PBS/ 2% FCS wash buffer.
   b. If the WBC count is <2 x 10^3 cells/µL, the sample must be concentrated prior to washing.
2. For each sample add 100 µL of whole blood or bone marrow specimen to a 12 x 75 mm test tube.
3. Add 3.0 mL of the PBS/ 2% FCS wash buffer. Mix by gentle inversion.
4. Centrifuge at 1000 x g for 2 minutes.
5. Aspirate and discard supernatant.
6. Repeat steps 3 to 5 two additional times.
7. Resuspend the washed pellet in either PBS/ 2% FCS or PBS/ 50% mouse serum to the initial 100 µL volume.
8. Proceed to Staining Procedure

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IG-720–RUO_L_AO

BECKMAN COULTER

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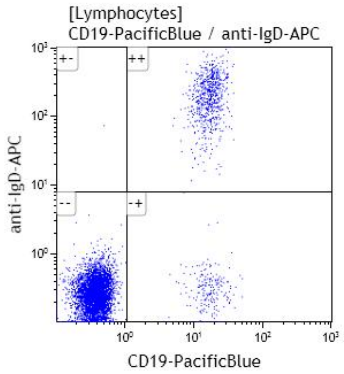
EXAMPLE DATA

The histograms below is a biparametric representation of a lysed normal whole blood. The staining is performed with the following Anti-Human IgD-APC
Acquisition is with a Navios flow cytometer, using CXP acquisition software and Kaluza analyse software.



Pre-washed lysed normal whole blood sample, gated on lymphocytes

SELECTED RESEARCH REFERENCES

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2. Geisberger, R., Lamers, M. and Achatz, G. The riddle of the dual expression of IgM and IgD, Immunology, 2006, 118, 429–437
3. Muramatsu, M., Kinoshita, K., Fagarasan,S., Yamada,S., Shinkai, Y., and Honjo, T. Class Switch Recombination and Hypermutation Require Activation-Induced Cytidine Deaminase (AID), a Potential RNA Editing Enzyme, Cell, 2000, 102, 553–563.
4. Chen, K et al. Immunoglobulin D enhances immune surveillance by activating antimicrobial, pro-inflammatory and B cell-stimulating programs in basophils, Nat Immunol. 2009; 10(8): 889–898.
5. Weller, S., Reynaud, C-A., and Weill, J-C., Splenic marginal zone B cells in humans: Where do they mutate their Ig receptor? Eur. J. Immunol. 2005. 35: 2789–2792.
6. Ohta, Y. and Flajnik, M., IgD, like IgM, is a primordial immunoglobulin class perpetuated in most jawed vertebrates, PNAS, 2006, 103, 10723–10728.

TRADEMARKS

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