IOTest Anti-Human IgM heavy chain-APC

PN B30654 – 50 tests – Liquid – 10 µL/test – Clone SA-DA4

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

SPECIFICITY

The anti-IgM SA-DA4 monoclonal antibody (mAb) binds specifically to mu heavy chain of Human Immunoglobulin (IgM) (1, 2, 3, 4).

The anti-IgM mAb binds to circulating IgM as well as to IgM antibodies bound to the FcµR. The FcµR is a transmembrane single sialoglycoprotein of 60 kD with O-linked oligosaccharides (1, 2). It contains an extracellular Ig-like domain homologous to two other IgM-binding receptors (polymeric Ig receptor and Fc α / µR) but exhibits an exclusive Fcµ-binding specificity. The cytoplasmic tail of FcµR contains conserved Ser and Tyr residues, but none of the Tyr residues match the immunoreceptor tyrosine-based activation, inhibitory, or switch motifs. Unlike other FcRs, the major cell types expressing FcµR are adaptive immune cells, including B and T lymphocytes. After antigen-receptor ligation or phorbol myristate acetate stimulation, FcµR expression was up-regulated on B cells but was down-modulated on T cells, suggesting differential regulation of FcµR expression during B and T cell activation (5).

The FcµR can be expressed as a cell surface activation antigen throughout the pre-B and B cell stages in differentiation (6, 7). Receptor expression is not directly linked with IgM production, as both µ- pre-B cells and isotype-switched B cells may express the FcµR. The receptor molecules produced by both pre-B and B cells are identical in size and are characterized as an acidic sialoglycoprotein with O-linked oligosaccharide (8). The FcµR is thus the third member of a family of Fc receptors expressed on B-lineage cells, and its preferential expression on activated B cells suggests a potential role in the response to antigens.

FcµR has no inhibitory activity in Fas-mediated apoptosis and that such inhibition is only achieved when anti-Fas antibody of an IgM but not IgG isotype is used for inducing apoptosis.

Given that IgM antibody is a first line of host defense, it is reasonable to propose that FcµR may contribute to enhancement of B cell responses by interacting with BCR (B cell receptor) (9, 10). Another potential role for FcµR on B cells is antigen presentation.

It seems possible that FcµR on T cells may interact with the IgM BCR or IgM/antigen complexes on B cells to facilitate T and B cell interactions, thereby enhancing B cell activation. FcµR may also trigger cytotoxic T cells in IgM antibody–dependent cell-mediated cytotoxicity.

REAGENT

IOTest Anti-Human IgM heavy chain-APC Conjugated Antibody
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Clone	SA-DA4
Isotype	IgG1, Mouse
Immunogen	Human immunoglobulin heavy-chain from myeloma cells
Hybridoma Source	X63 x balb/c
Purification	Purified
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

BECKMAN COULTER

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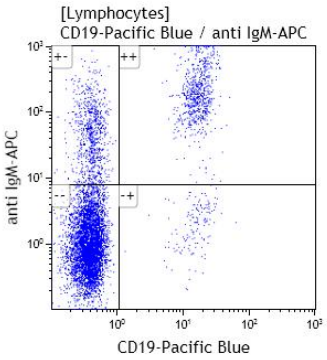
EXAMPLE DATA

The histograms below are biparametric representations of a lysed normal whole blood. The staining is performed with the following Anti-IgM-APC.

Acquisition is with a Navios flow cytometer, using CXP acquisition software.



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

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TRADEMARKS

MANUFACTURED BY:

IMMUNOTECH SAS
a Beckman Coulter Company
130, avenue de Lattre de Tassigny
B.P. 177 - 13276 Marseille Cedex 9
France

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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