IOTest® CD120a (TNFR1)-PE

PN A22359 – 100 tests – 20 µL / test – Clone H398

For Research Use Only. Not For Use In Diagnostic Procedures.

SPECIFICITY

The CD120a antigen, also known as tumor necrosis factor receptor type 1 (TNFR1) or p55 (1 – 3) belongs to the TNF receptor superfamily. Tumor necrosis factor (TNF) is a cytokine with a wide range of biological activities in inflammatory and immunologic responses. These activities are mediated by specific cell surface receptors of 55 kDa (CD120a) and 75 kDa (CD120b) apparent molecular masses. CD120a is the main receptor for TNF-α. Also able to bind to this receptor, although with lower affinity than TNF-α, are lymphotoxin (LT)-α (or LTA), also known as TNF-β when secreted as a homotrimer, as well as the membrane-associated LTα2/β1 heterotrimer. Crystallographic studies revealed that the complex has three receptor molecules bound symmetrically to one TNF-β trimer (4). TNF and LT are released by macrophages and certain lymphocytes, and are capable of promoting the lysis of many cell types including some tumor cells (5). CD120a is a type I transmembrane glycoprotein, containing four cysteine-rich subdomains in the extracellular portion and 4 potential N-linked glycosylation sites (1, 2). The cDNA encodes a protein divided into an extra-cellular domain of 189 residues, a trans-membraneous segment of 23 residues, and a cytoplasmic domain of 222 residues (1, 2).

CD120a has a unique domain called the death domain (DD), which is localized in the C-terminal portion of the cytoplasmic domain (6, 7). The trimerization of the CD120a induced by TNF results in the recruitment of TNFR1-associated death domain protein (TRADD) and RIP via the interaction with the death domain (9 – 11). Binding of ligands to CD120a can generate responses as diverse as apoptosis and the expression of NF-κB-dependent pro-survival genes and protection against TNF-induced apoptosis (12).

A soluble form of CD120a has also been described (13 – 14) which can be generated from a proteolytic cleavage of the cell surface forms and acts as inhibitor of TNF responses (14).

The CD120a is mainly expressed by monocytes / macrophages and neutrophils (1, 2, 15). It is also expressed on about 10% of bone marrow mononuclear cells (16), and on endothelial cells (17, 18). In the follicular apical light zone, follicular dendritic cells express transforming growth factor-beta receptor 2 (TGF-βR2), CD116, and CD120a (19). Finally, bronchoalveolar cells have also been described to express CD120a (20).

The CD120a H398 monoclonal antibody (mAb) has antagonistic properties (21). It reacts with the extracellular part of the CD120a. It also reacts with the soluble receptor.

REAGENT

IOTest CD120a (TNFR1)-PE Conjugated Antibody
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Clone	H398
Isotype	IgG2a, mouse
Immunogen	Purified TNFR1
Source	Ascites fluid
Purification	Affinity chromatography on Protein A
Conjugation	R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig.

Excitation wavelength: 488 nm
Maximum emission wavelength: 575 nm
Main emission color: Orange-red

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

Study of CD120a-positive cells by flow cytometry

STATEMENT OF WARNINGS

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 handled as if capable of transmitting infection 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.

STORAGE CONDITIONS AND STABILITY

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

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.
The use of Versalyse (PN IM3648) with concomitant fixation (e.g. using IOTest 3 Fixative Solution (PN IM3515), followed by a wash is required to yield optimal results.

Preparation of working solutions (quantity for 1 tube):

1. "Fix-and-lyse" mixture: freshly mix 1 mL of VersaLyse (PN IM3648) with 25 µL of undiluted IOTest 3 Fixative Solution (PN IM3515). Prepare a sufficient amount of the "fix-and-lyse" mixture for the total number of samples.
2. Fixing buffer: mix 6.25 µL of undiluted IOTest 3 Fixative Solution (PN IM3515) in 0.5 mL PBS. Prepare a sufficient amount of the fixing buffer for the total number of samples.

NOTE: Unlike what is stated on the package insert of the IOTest 3 Fixative Solution (PN IM3515), the present procedure does not use this fixative solution as a 10X concentrated solution.

Procedure:

1. Label tubes for analysis.
2. Pipet into each tube 10 µL of the monoclonal antibody (mAb) or mAb mixture.
3. Add 100 µL of whole blood.
4. Vortex each tube for 5 seconds.
5. Incubate at room temperature (18 – 25°C) for 20 minutes. Protect from light.
6. Add 1 mL of the "fix-and-lyse" mixture to each tube and vortex immediately for one second after each addition.
7. Incubate at room temperature for at least 10 minutes. Let tubes sit, protected from light.
8. Centrifuge the tubes at 150 x g for 5 minutes and discard the supernatant by aspiration.
9. Add 3 mL of PBS.
10. Centrifuge the tubes at 150 x g for 5 minutes and discard the supernatant by aspiration.
11. Resuspend the pellets by addition of 0.5 mL of fixing buffer.
12. Vortex each tube for 5 seconds.
13. Store at 2 – 8°C until analysis:
    a) for fresh specimens (<12 hours), analyze within 6 hours;
    b) for older specimens, analyze within 2 hours.

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BECKMAN COULTER

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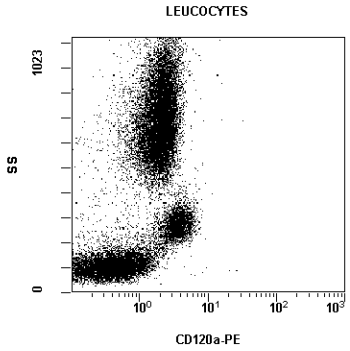
EXAMPLE DATA

The histogram below is a biparametric representation, side scatter (SS) *versus* fluorescence intensity of a lysed normal whole blood sample. Staining is with CD120a (TNFR1)–PE (PN A22359).

Acquisition is with a COULTER® EPICS® XL™ flow cytometer. Analysis is with the CXP analysis software.



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PRODUCT AVAILABILITY

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PE is licensed under patent 4,520,110

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TRADEMARKS

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