BD Horizon™

Technical Data Sheet

BV750 Mouse Anti-Human TNF

Product Information

Material Number: 566359
Alternate Name: Tumor necrosis factor alpha; TNF-α; TNF-α; TNFSF2; Cachectin
Size: 50 µg
Concentration: 0.2 mg/ml
Clone: MAb11
Immunogen: Recombinant Human TNF
Isotype: Mouse IgG1, κ
Reactivity: QC Testing: Human
Reported Reactivity: Rhesus, Cynomolgus, Baboon

Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MAb11 monoclonal antibody specifically binds to human tumor necrosis factor (TNF, also known as TNF-α) protein. TNF is an efficient juxtacrine, paracrine and endocrine mediator of inflammatory and immune functions. It regulates the growth and differentiation of a variety of cell types. TNF is cytotoxic for transformed cells when in conjunction with IFN-γ. It is secreted by activated monocytes/macrophages and other cells such as B cells, T cells and fibroblasts. The immunogen used to generate the MAb11 hybridoma was recombinant human TNF. The MAb11 antibody has been reported to crossreact with Rhesus Macaque TNF.

The antibody was conjugated to BD Horizon BV750 which is part of the BD Horizon Brilliant™ Violet family of dyes. This dye is a tandem fluorochrome of BD Horizon BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 750-nm. BD Horizon Brilliant BV750 can be excited by the violet laser (405 nm) and detected with a 750/30 nm filter with a 740 nm long pass. Due to spectral differences between labeled cells and beads, using BD™ CompBeads can result in incorrect spillover values when used with BD Horizon BV750 reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended.

Flow cytometric analysis of TNF expression in stimulated human peripheral blood lymphocytes. Human peripheral blood mononuclear cells were stimulated for 6 hours with Phorbol 12-Myristate 13-Acetate (Sigma P-8139; 50 ng/ml final concentration) and Ionomycin (Sigma I-0634; 1 µg/ml final concentration) in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) (Cat. No. 554724). The cells were harvested, washed with BD Pharmedigm™ Stain Buffer (FBS) (Cat. No. 554656), and fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655). The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with either BD Horizon™ BV750 Mouse IgG1 κ Isotype Control (Cat. No. 566360; Left Plot) or BD Horizon BV750 Mouse Anti-Human TNF antibody (Cat. No. 566359; Right Plot) at 0.5 µg/test. Two-color flow cytometric dot plots showing the correlated expression of TNF (or Ig Isotype control staining) versus Autofluorescence were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD LSRFortessa™ X-20 Cell Analyzer System. Data shown on this Technical Data Sheet are not lot specific.

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Preparation and Storage
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with BD Horizon BV750 under optimum conditions, and unconjugated antibody and free BD Horizon BV750 were removed.

Application Notes

Application

| Intracellular staining (flow cytometry) | Routinely Tested |

Recommended Assay Procedure:
For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349).

Suggested Companion Products

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<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
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<tr>
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<td>Fixation Buffer</td>
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<td>555061</td>
<td>HiCK-1 Human Cytokine Positive Control Cells</td>
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<tr>
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<td>Brilliant Stain Buffer</td>
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<td>Perm/Wash Buffer</td>
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<tr>
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<td>Brilliant Stain Buffer</td>
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<td>Stain Buffer (BSA)</td>
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Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
6. BD Horizon Brilliant™ Violet 750 is covered by one or more of the following US patents: 8,158,444; 8,802,450; 8,575,303; 8,455,613; 8,227,187; 8,841,072; 8,110,673.
7. An isotype control should be used at the same concentration as the antibody of interest.

References
Smith RA, Bagliro C. The active form of tumour necrosis factor is a trimer. J Biol Chem. 1987; 262(15):6951-6954. (Biology)