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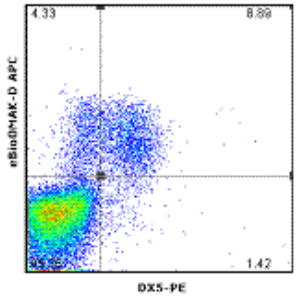
Anti-Mouse Perforin APC

Catalog Number: 17-9392
RUO: For Research Use Only



Intracellular staining of C57BL/6 splenocytes cultured with recombinant mouse IL-2 for 4 days with Anti-Mouse CD49b (Integrin α2) PE (cat. 12-5971) and 0.5 µg of Anti-Mouse Perforin APC. Cells in the lymphocyte gate were used for analysis.

Product Information

Contents: Anti-Mouse Perforin APC
REF Catalog Number: 17-9392
Clone: eBioOMAK-D
Concentration: 0.2 mg/ml
Host/Isotype: Rat IgG2a, κ

Formulation: aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer
Temperature Limitation: Store at 2-8°C. Do not freeze. Light sensitive material.
LOT Batch Code: Refer to Vial
Use By: Refer to Vial
Caution, contains Azide

Description

The eBioOMAK-D antibody reacts with mouse perforin (pore-forming protein, pfp, Prf). Perforin is one of the cytolytic mediators present in the cytoplasmic granules of cytotoxic T lymphocytes (CTL) and natural killer cells (NK). Perforin is involved in the killing function by CTLs and NKs and has an important role in the immune response against tumors and virus infections.

By immunobloting, eBioOMAK-D recognizes a ~70kDa band in lysates of CTLL-2 mouse cytotoxic cell line and in lysates of IL-2 stimulated but not unstimulated mouse splenocytes. By multi-color intracellular flow cytometric analysis, eBioOMAK-D staining is increased upon stimulation (IL-2 or anti-CD3/28). Intracellular flow staining results showing upregulation of protein expression have been confirmed by immunoblotting. Furthermore, stimulated Perforin Knock-out (developed by Walsh) splenocytes do not stain with eBioOMAK-D nor is any protein detectable by western blotting with eBioOMAK-D as well as other anti-mouse perforin antibodies. Please note that the Kagi perforin knock-out mice may synthesize a truncated form of the protein which may be recognized by eBioOMAK-D.

In IL-2 stimulated mouse splenocytes, NK cells (as determined by CD49b staining) contain perforin while CD8 cells contain little to none and can vary with culture conditions. This has been confirmed by staining and western blotting the two populations using both OMAK-D and P1-8 antibodies. In contrast stimulation of splenocytes with anti-CD3/CD28 antibodies does result in an increase of perforin on both NK cells and CD8 cells.

eBioOMAK-D is also crossreactive to human perforin and co-stains CD56 positive cells in PBMC.

Expression of perforin and Granzyme B do not always correlate (as discussed above in the CD8 population of IL-2 stimulated splenocytes). Granzyme B typically is expressed earlier and at higher levels. Expression of Granzyme B is dramatically increased (more than 10,00 fold based on mRNA estimates and significantly at the protein level based on western blotting and flow analysis) compared to a minimal increase (10-100 fold) in perforin mRNA and protein with IL-2 stimulation.

For intracellular staining and flow cytometric analysis with direct conjugates of anti-mouse perforin, it is highly recommended to use the Foxp3 buffer system (cat. 00-5523). Other buffers may yield varying results. For more information, please contact technical support at tech@ebioscience.com.

Applications Reported

This eBioOMAK-D antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested

This eBioOMAK-D antibody has been tested . This can be used at less than or equal to 1 µg per test. A test is defined as the amount (µg) of

antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

References

Fehniger TA, Cai SF, Cao X, Bredemeyer AJ, Presti RM, French AR, Ley TJ. Acquisition of Murine NK Cell Cytotoxicity Requires the Translation of a Pre-existing Pool of Granzyme B and Perforin mRNAs Immunity 2007 May (Epub) (eBioOMAK-D, IC, PubMed)

Liu CC, Walsh CM, Young JD. Perforin: structure and function. Immunol Today. 1999. 16(4):194-201.

Opferman JT, Ober BT, Ashton-Rickardt PG. Linear differentiation of cytotoxic effectors into memory T lymphocytes. Science. 1999. 283 (5408):1745-8.

Slifka MK, Rodriguez F, Whitton JL. Rapid on/off cycling of cytokine production by virus-specific CD8+ T cells. Nature. 1999. 401(6748):76-9.

Walsh CM, Matloubian M, Liu CC, Ueda R, Kurahara CG, Christensen JL, Huang MT, Young JD, Ahmed R, Clark WR. Immune function in mice lacking the perforin gene. Proc Natl Acad Sci U S A. 1994. 91(23):10854-8.

Related Products

12-8822 Anti-Mouse Granzyme B PE (16G6)
12-9994 Anti-Human Perforin PE (dG9 (delta G9))
17-4321 Rat IgG2a K Isotype Control APC

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