Technical Data Sheet

V450 Mouse Anti-Human IFN-α[2b]

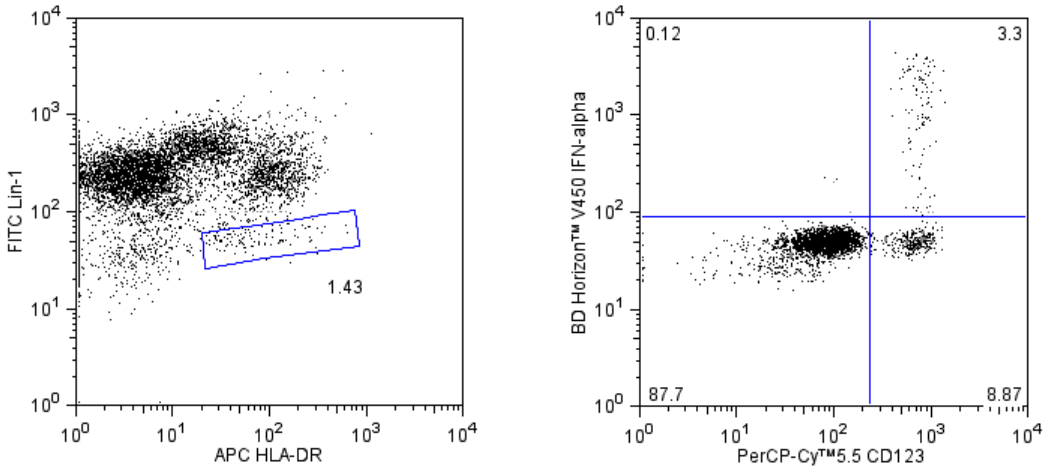
Product Information

Material Number:	**561382**
Alternate Name:	IFNa, IFNα
Size:	50 tests
Vol. per Test:	5 µl
Clone:	7N4-1
Immunogen:	Human IFN-α2b
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The 7N4-1 antibody reacts with human IFN-α2b and to a lesser extent with IFN-α7. It does not react with IFN-α1 nor IFN-α4. IFN-α2b is one of the three variants of IFN-α2 that have been isolated from human cell lines. IFN-α2b is the variant predominantly produced by human leukocytes. Human IFN-α2b belongs to the IFN-α class of proteins also known as leukocyte interferons. IFN-α comprises a family of related but distinct proteins with molecular weights ranging from 16-27 kDa with antiviral, antiproliferative and immunomodulatory activities. The IFN-α family is composed from as many as 14 different genes. The immunogen used to generate the 7N4-1 hybridoma was E. coli-expressed recombinant human IFN-α2b. This is a neutralizing antibody.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser Ex max of 406 nm and has an Em Max at 450 nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



Multicolor flow cytometric analysis of IFN-alpha2b in stimulated peripheral blood mononuclear cells (PBMC). Human PBMC were stimulated using CpG oligodeoxynucleotide (Coley Pharmaceutical Co., Cat. No. 2336) and then treated with BD FastImmune™ Brefeldin A (BFA) Solution (Cat. No. 347688). Cells were harvested, washed, and stained with FITC-conjugated Lin-1 Cocktail (Cat. No. 340546), PerCP-Cy™5.5 Mouse Anti-Human CD123 (Cat. No. 558714/560904), and APC Mouse Anti-Human HLA-DR (Cat. No. 559868) simultaneously. The cells were then fixed and permeabilized (see Recommended Assay Procedure) followed by intracellular staining with BD Horizon™ V450 Mouse Anti-Human IFN-α2b (IFN-alpha2b; Cat. No. 561382). The two-color flow cytometric dot plots show the expression of CD123 versus IFN-alpha (Right Panel) derived from gated events with the light scattering characteristics of lymphocytes and monocytes and the immunofluorescence characteristics of Lin-1 negative and HLA-DR positive cells (Left Panel). Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with BD Horizon™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:
PROCEDURE

Cell Activation

Note: The following cellular activation procedure needs to be performed in a tissue culture hood using aseptic technique.

1. Isolate fresh human peripheral blood mononuclear cells (PBMC) from 60 ml of fresh blood, and wash twice with sterile 1X Dulbecco's Phosphate Buffered Saline (PBS). Centrifuge the cells at 250*g* for 10 minutes and discard the supernatant.
2. Suspend the cells in complete tissue culture medium (RPMI supplemented with 1% Pen/Strep, 1% L-glutamine and 10% fetal bovine serum). Count and adjust the cell concentration to 2 to 3 million cells/mL.
3. Label two sterile 50-mL conical tubes as "Not Stimulated" and "Stimulated with CpG." Dispense PBMC to each tube (2 million cells/test)
4. Add 5 µg of CpG oligodeoxynucleotide (Coley Pharmaceutical Co. Cat. No. 2336) per ml of cells to the "Stimulated with CpG" tube. Cap the tube and vortex gently.
5. Incubate both tubes for 2 hours at 37°C.
6. Dilute BD FastImmune™ Brefeldin A (BFA) Solution (Cat. No. 347688) 1 to 10 in sterile 1X PBS.
7. Add 20 µl of 1X BFA per mL to both tubes ("Not Stimulated" and "Stimulated with CpG"). Incubate tubes at 37°C for 2 hours.

Note: Keep both CPG and BFA aliquots at -20°C.

8. Add 100 µl of 20 mM EDTA (Sigma Cat. No. E7889) per mL of cells to both tubes and incubate overnight at 4°C.

Surface and Intracellular Staining

9. Centrifuge the two tubes at 250*g* for 10 minutes. Discard the supernatants by aspiration and resuspend cells in 25 mL of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656).
10. Centrifuge the tubes at 250*g* for 10 minutes. Discard the supernatants and resuspend cells in Stain Buffer (FBS) at 20 million per ml.
11. Label 12 × 75 mm polypropylene tubes appropriately. Add 100 µl of cells to each tube.
12. Add surface staining antibodies to each tube: Human Lin-1 FITC (Cat. No. 340546), Human CD123 PerCPCy5.5 (Cat. No. 558714/560904), and Human HLA-DR APC (Cat. No. 559868). Incubate the tubes at room temperature for 30 minutes in the dark.
13. Following incubation, add 2 mL of cold Stain Buffer (FBS) to each tube and centrifuge at 250*g* for 10 minutes. Discard the supernatants by aspiration and vortex the pellets to resuspend the cells.
14. Add 1 mL of room temperature BD Cytofix/Cytoperm Buffer (Cat. No. 554722) to each tube. Mix well and incubate at room temperature in the dark for 30 minutes.
15. Add 2 mL of cold BD Perm/Wash™ Buffer (Cat. No. 554723) to each tube and centrifuge at 500*g* for 5 minutes; discard the supernatants by aspiration and vortex the pellets to resuspend cells. Repeat the wash step again.
16. Add the fluorescent intracellular staining antibody, BD Horizon™ V450 Mouse Anti-Human IFN-α2b (IFN-alpha2b; Cat. No. 561382; 5 µl/test) or the proper fluorescent immunoglobulin isotype control at the appropriate volume per test to the tubes and mix well by vortexing. Bring test volume to 100 µl using cold BD Perm/Wash Buffer. Incubate tubes at room temperature for 60 minutes in the dark.
17. Add 2 mL of cold BD Perm/Wash Buffer to each tube and centrifuge at 500*g* for 5 minutes; discard supernatant by aspiration and vortex pellet to suspend cells.
18. Add 500 µl of cold Stain Buffer to each tube for immediate flow cytometric analysis.

Optional: Resuspend the cell pellets with 200 µl of cold 1% -formaldehyde solution and keep the tubes at 4°C in the dark up to 24 hours before flow cytometry. If storing longer than 24 hours, we recommend washing cells in Stain Buffer (FBS) as extended incubation with fixatives might affect fluorochromes.

Flow Cytometry and Data Analysis

Acquire at least 500,000 events (lymphocytes and monocytes).

A sequential gating strategy is required for successful data analysis:

1. Gate tightly on the lymphocytes and monocytes using the forward- and side-light scatter profiles.
2. View the Lin-1 versus HLA-DR profile of the gated lymphocytes and monocytes, and select the Lin-1-negative, HLA-DR-positive population. Be sure not to include any of the Lin-1-positive cells.
3. View the IFN-alpha2b (IFN-alpha) versus CD123 profile of the gated cells to detect the IFN-alpha-positive cell population.

The "Not Stimulated" sample is used as a negative control that can be used to set markers for measuring the frequency of IFN-α2b-positive (IFN-alpha-positive) cells.

Open up the second gate on Lin-1- HLA-DR+ cells and from there open up the third gate for CD123+ IFN-alpha+ cells. Acquire about 150 to 200 events in the CD123+ IFN-alpha+ double positive cell quadrant.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554722	Fixation and Permeabilization Solution	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
558714	PerCP-Cy™5.5 Mouse anti-Human CD123	100 tests	7G3
560904	PerCP-Cy™5.5 Mouse Anti-Human CD123	25 tests	7G3
559868	APC Mouse Anti-Human HLA-DR	100 tests	TU36

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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 Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
6. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.

References

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