Technical Data Sheet

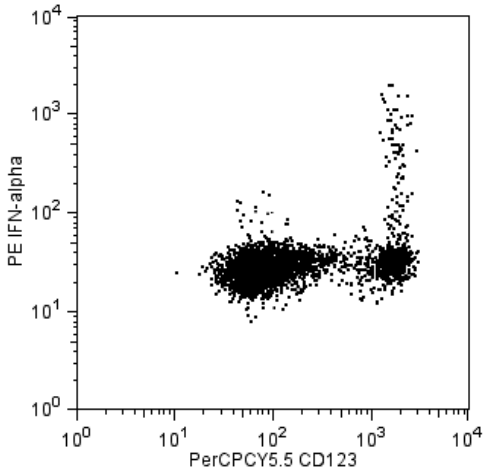
PE Mouse anti-Human IFN-α[2b]

Product Information

Material Number:	**560097**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	7N4-1
Immunogen:	E. coli-expressed recombinant human IFN-α2b
Isotype:	Mouse IgG1, κ
Reactivity:	QC Tested: Human
Storage Buffer:	Aqueous buffered solution containing BSA 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.



Flow cytometric analysis of PE anti-human IFN alpha on stimulated PBMC. *Human PBMC were stimulated using CPG oligodeoxynucleotide and then treated with BFA. Cells were then stained with FITC conjugated Lin-1 cocktail, PerCP-Cy™5.5 Mouse anti-Human CD123, and APC Mouse Anti-Human HLA-DR simutaneously. Cells were fixed and permeabilized (see recommended assay procedure) followed by intracellular staining with PE anti-human IFN-alpha. The dot plots were derived from the gated events based on light scattering characteristics of lymphocytes and fluorescence characteristics of Lin-1 negative, HLDA-DR positive shown as IFN-alpha vs CD123. Flow cytometry was performed on a BD FACSCalibur™ System. Please refer to Recommended Assay Procedure for a full protocol and list of materials*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were 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

Please refer to chart in Suggested Companion Products section for names and sources of materials used in the following protocol

Cell Activation

Note: The following procedures need to be performed in the hood using aseptic technique.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

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1. Isolate fresh human peripheral blood mononuclear cells PBMC from 60ml of fresh blood, and wash 2X with sterile 1 X PBS. Centrifuge the cells at 250 Xg for 10 minutes and discard the supernatant.
2. Suspend the cells in complete medium (RPMI supplimented with 1% Pen/Strep, 1% L-glutamine and 10% FBS). Count and adjust the cell concentration to 2-3 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/ml).
4. Add 5µg of CPG oligodeoxynucleotide 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) 1 to 10 in sterile 1 X PBS.
7. Add 20µl of 1X BFA per ml to both tubes (stimulated and unstimulated). Incubate tubes at 37°C for 2 hours.
   Note: Keep both CPG and BFA aliquots at -20°C.
8. Add 100µl of 20 mM EDTA per mL of cells to both tubes and incubate overnight at 4°C.

Surface and Intracellular Staining

9. Centrifuge the two tubes at 250 X g for 10 minutes. Discard the supernatants by aspiration and re-suspend cells in 25 ml of BD FACS Wash Buffer
10. Centrifuge the tubes at 250 X g for 10 minutes. Discard the supernatants and re-suspend cells in FACS Wash buffer at 20 million cells/ml.
11. Label 12x75mm polypropylene tubes appropriately. Add 100 µl of cells (2 million cells/test) to each tube.
12. Add surface staining antibodies to each tube (human Lin-1 FITC, human CD123 PerCPCy5.5, and human HLA-DR APC or PE conjugate). Incubate the tubes at room temperature for 30 minutes in the dark.
13. Following incubation, add 2 ml of cold FACS Wash Buffer to each tube and centrifuge at 250 X g for 10 minutes. Discard the supernatants by aspiration and vortex the pellets to re-suspend the cells.
14. Add 1 ml of room temperature BD Cytofix/Cytoperm Buffer 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 to each tube and centrifuge at 500 X g for 5 minutes; discard the supernatants by aspiration and vortex the pellets to re-suspend cells.
16. Add intracellular staining antibody anti-human IFN-alpha (20µl/test) or the proper isotype control at the appropriate volume per test to the tubes and mix well by vortexing. Bring test volume to 100µl 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 X g for 5 minutes; discard supernatant by aspiration and vortex pellet to suspend cells.
18. Add 300µl of cold BD Perm/Wash Buffer to each tube for immediate flow cytometric analysis.

Optional: Re-suspend the pellets with 200µl of cold 1% -formaldehyde 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 wash buffer 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:

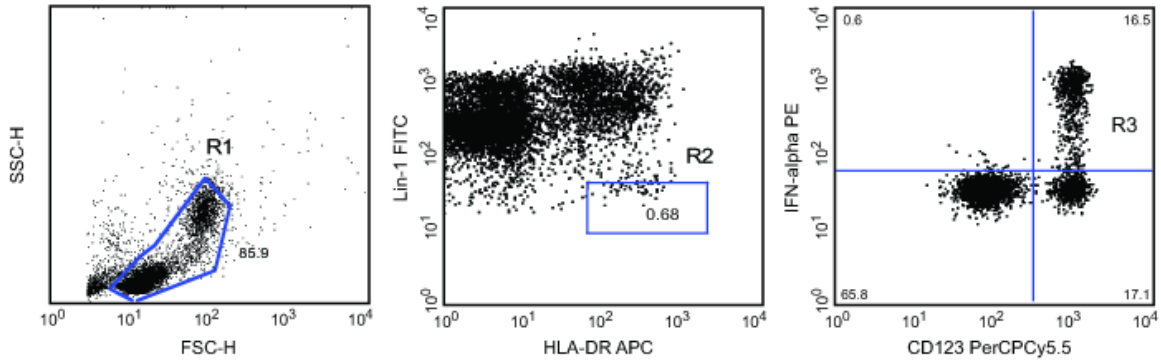
1. Gate tightly on the lymphocytes and monocytes using scatter profiles.
2. View the Lin-1 versus HLA-DR profile of the gated lymphocytes and monocytes, and select the Lin-1-negative, HLA-DR-positive population (R2 in the figure). Be sure not to include any of the Lin-1-positive cells.
3. View the IFN- versus CD123 profile of the gated cells to detect the IFN- -positive population.

Unstimulated sample is used as a negative control could also be used to set the markers.

Open up the second gate on Lin-1- HLA-DR+(R2) and from there open up the third gate for CD123+ IFN-alpha+(R3). Acquire about 150-200 events in CD123+ IFN-alpha+ double positive quadrant (R3). See below for correct and incorrect gating examples.

The proper gating for IFN-alpha is crucial for proper analysis. Below are two examples. The first example is how we recommend gating for proper detection of IFN-alpha. The second example is the **incorrect** way to gate for IFN-alpha.

Example of **correct** gating strategy for detecting IFN-alpha in CPG stimulated PBMC

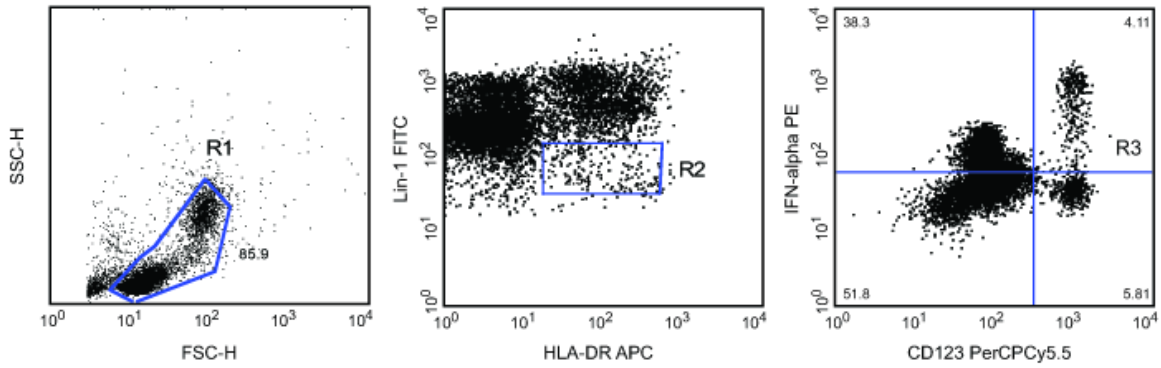


R1= Activated PBMC
(gating lymphocytes and monocytes)

R2=Lin-1 FITC$^-$ HLA-DR APC$^+$

Double positive cells can be seen in R3

Example of **incorrect** gating strategy for detecting IFN-alpha in CPG stimulated PBMC



R1= Activated PBMC
(gating lymphocytes and monocytes)

R2=Lin-1 FITC$^-$ HLA-DR APC$^+$

Double positive cells can be seen in R3

Suggested Companion Products

Reagent	Clone	Cat. No.	Storage	Vendor
Cytofix/Cytoperm™	NA	554722	4^0C	BD
Perm/Wash Buffer 10X	NA	554723	4^0C	BD
Brefeldin A (BFA)	NA	347688	-20^0C	BD
Stain Buffer (FBS)	NA	554656	4^0C	BD
EDTA, 0.5M	NA	E7889	4^0C	Sigma-Aldrich
CPG Oligodeoxynucleotide (CPG ODN)	NA	2336	-20^0C	Coley Pharmaceutical Company
RPMI-1640	NA	SH30096	4^0C	HyClone
Lin-1 FITC	NA	340546	4^0C	BD
PerCP-Cy™5.5 Mouse anti-Human CD123	7G3	558714	4^0C	BD
APC Mouse Anti-Human HLA-DR	TU36	559868	4^0C	BD
PE Mouse anti-Human IFN-α	7N4-1.2	560097	4^0C	BD
PE Mouse IgG1, κ Isotype Control	MOPC-21	555749	4^0C	BD

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. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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