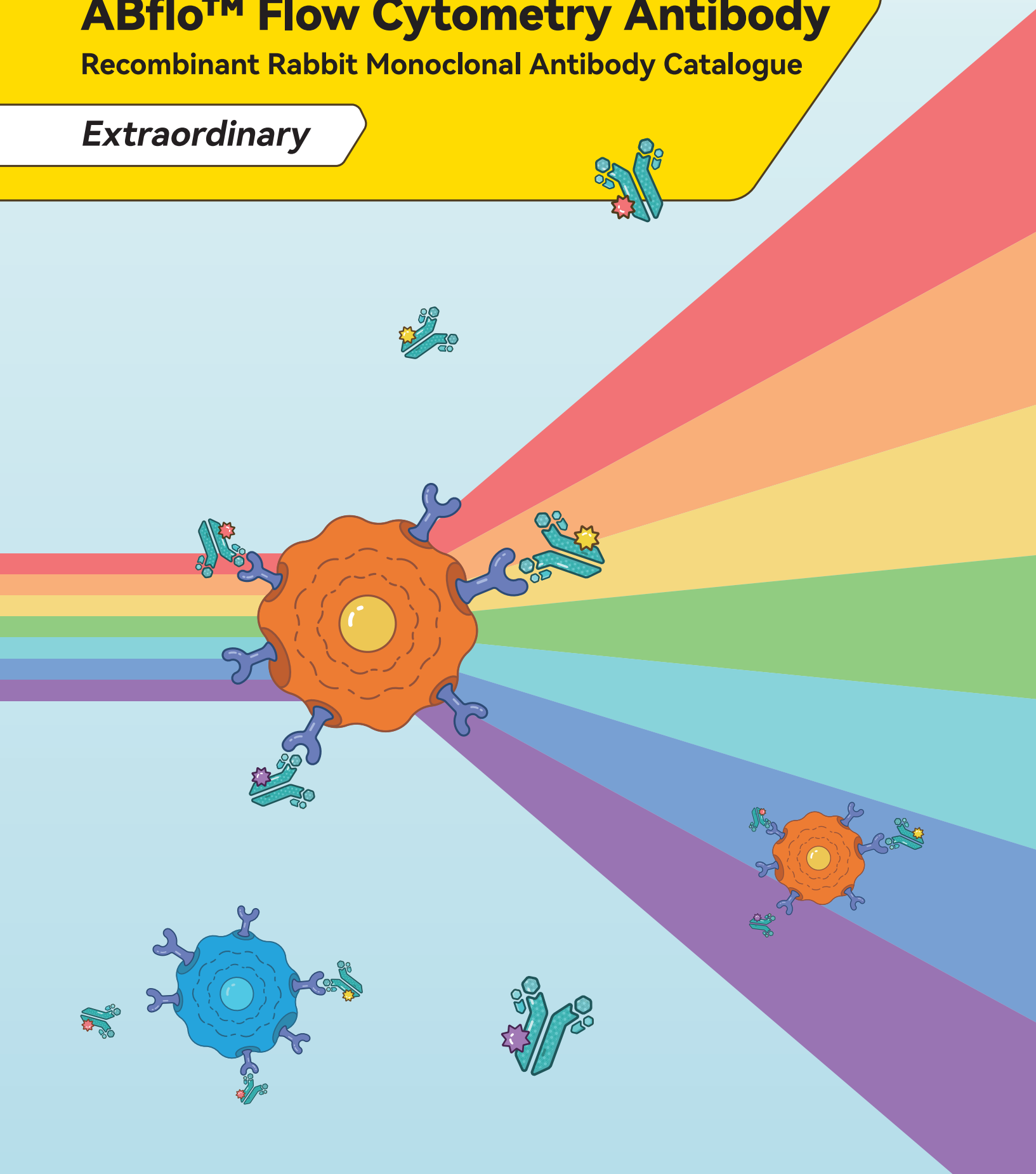
ABclonal Technology®

ABflo™ Flow Cytometry Antibody

Recombinant Rabbit Monoclonal Antibody Catalogue

Extraordinary



ABflo™ Flow Cytometry Antibody

Flow cytometry (FCM) employs flow cytometers and high-quality flow cytometry antibodies as essential tools for conducting rapid, multi-parameter quantitative analysis and sorting of cells or micro-particles in a fast linear flow state. Leveraging the groundbreaking SMab™ recombinant rabbit monoclonal antibody platform, ABclonal has successfully developed a series of innovative and superior rabbit monoclonal flow cytometry antibodies known as ABflo™. These antibodies showcase exceptional attributes including remarkable specificity, sensitivity, and consistent performance across batches. ABflo™ facilitates highly efficient cell analysis and sorting, delivering outstanding signal-to-noise ratios.

ABflo™ flow cytometry antibodies are offered in a variety of fluorescence labeling systems, encompassing proprietary ABflo™ fluorochrome conjugates as well as classic fluorochrome conjugates. These antibodies are provided in both unconjugated and classic clone flow cytometry formats, extending their versatility and potential applications. Additionally, they exhibit reactivity towards multiple species, including Human, Mouse, Pig, Dog, Cat, and Monkey, allowing for comprehensive coverage of various targets such as TBNK core targets, hematopoietic stem cell targets, conventional flow cytometry targets, and small molecules. This broad range of capabilities ensures the fulfillment of diverse requirements across various usage scenarios.

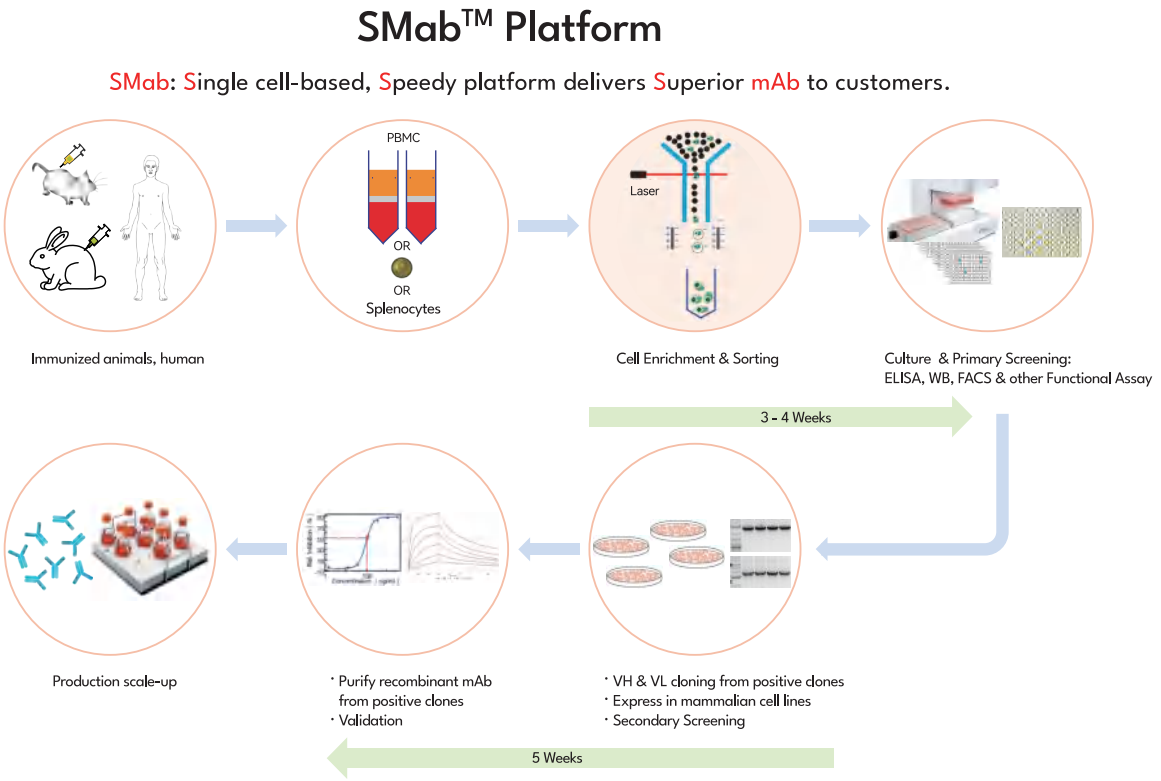
CONTENTS

1. ABclonal Flow Cytometry Antibody Development Platform

1.1 SMab™ Recombinant Monoclonal Antibody Discovery Platform

The SMab™ Recombinant Monoclonal Antibody Discovery Platform, abbreviated as SMab™, is an independent intellectual property-based platform for monoclonal antibody discovery. It bases on the single B cell technologies to screen and cultivate individual B cells, forming the foundation of this technology platform. The core of this platform lies in the integration of state-of-the-art immunological techniques with cell sorting technologies, enabling the selection of specific B lymphocytes from immunized animals. These selected B lymphocytes are then cultured in optimized media for several weeks to stimulate their proliferation and secretion of a quantity of antibodies for primary screening.

Following this, the genes encoding the obtained antibodies are amplified using cutting-edge molecular biology techniques. The amplified genes are subsequently used for large-scale recombinant monoclonal antibody production through *in vitro* expression. The primary objective of this platform is to provide high-quality monoclonal antibodies to researchers worldwide, as well as to large pharmaceutical companies, biotechnology companies, and enterprises engaged in clinical diagnostic reagent development.



1.2 Advantages of Recombinant Rabbit Monoclonal Antibodies for Flow Cytometry

ABflo™ is a novel recombinant rabbit monoclonal antibody for flow cytometry that surpasses traditional mouse monoclonal antibodies in performance.

Selected Clones, Excellent Performance

A rigorous process of high-quality antigen immunization is conducted, and thorough benchmarking against competitor detection is performed to ensure the selection of superior clones for production. Leveraging the distinct evolutionary relationship between rabbits and mice or humans, the development targeting human and mouse proteins benefits from a more robust immune response. This leads to the generation of high-performance antibodies, showcasing significant advantages over traditional mouse monoclonal antibodies.

Good Compatibility, High Reproducibility

Consistent results are attained through analysis and sorting using mainstream flow cytometry instruments alongside a diverse range of compatible reagents; exhibit high levels of consistency, provide reliable cross-platform data, and demonstrate excellent result transferability.

Comprehensive Validation, Rigorous Data

Thorough sample validation encompassing diverse samples and multicolor combinations, precise determination of optimal concentrations, exceptional inter-batch consistency, and remarkable stability.

Stable Supply Chain, Guaranteed Availability

A stable supply chain guarantees manageable delivery lead times, convenience, speed, safety, and the reliability of supply. It provides flexibility to accommodate custom production and labeling requirements for our existing product range.

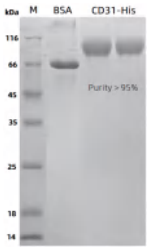
1.2.1 Selected Clones, Excellent Performance

SMab™ recombinant rabbit monoclonal antibodies for flow cytometry are generated using various types of high-quality antigens, including premium grade active proteins, stable cell lines, and DNA. This comprehensive approach ensures the development of top-tier antibodies and enables a diverse selection. Each antibody undergoes meticulous benchmarking against established flow cytometry brands, with only the superior or comparable clones being chosen for production.

Moreover, in the development of antibodies targeting human and mouse proteins, the distant evolutionary relationship between rabbits and mice or humans enables a more robust immune response, resulting in the generation of high-performance antibodies that offer significant advantages over traditional mouse monoclonal antibodies.

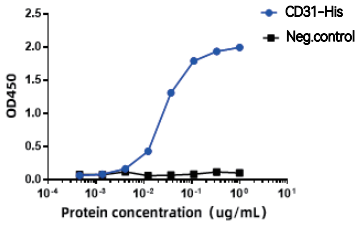
High-quality antigens - the key to successful antibody development

Immunization with CD31 Active Protein



SDS-PAGE

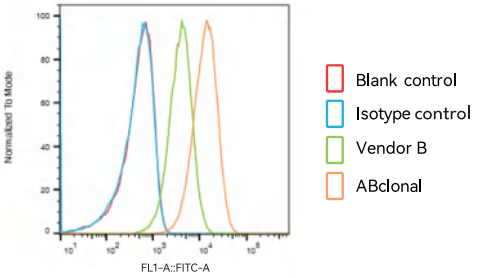
Results : Recombinant human CD31 protein was detected using SDS-PAGE and Coomassie Brilliant Blue staining, showing a distinct target band in the 85-105 kDa range, with a protein purity greater than 95%.



Bioactivity-ELISA

Results: Mouse anti-human CD31 antibody (1 μg/mL, 25 μL/well) was able to bind to human CD31 protein, with a linear range of 24.34-29.48 ng/mL.

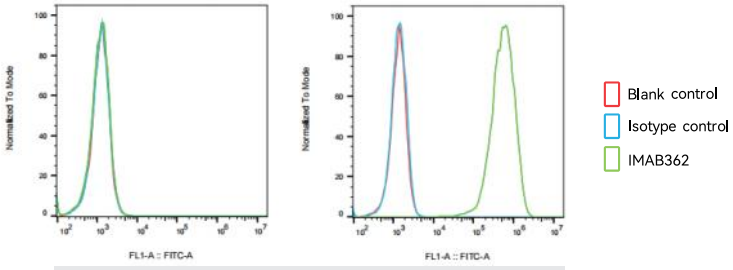
Human CD31-Cat.No.A22508



HEL cells

Results: Antibodies obtained from immunization with CD31 active protein showed significantly improved signal transition in flow cytometry under the same experimental conditions compared to competitors.

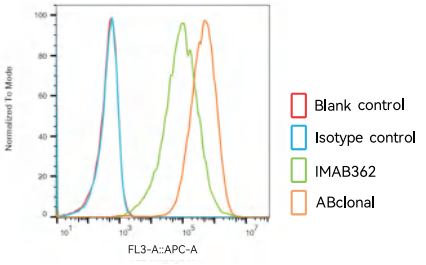
Immunization with Claudin 18.2 Stable Cell Line



Stable cell line: immunogen

Results: Flow cytometry analysis of stable cell lines demonstrated a signal transition that was 2.4 orders of magnitude higher compared to control cells, indicating the successful generation of stable cell lines.

Human Claudin 18.2-Cat.No.A23392

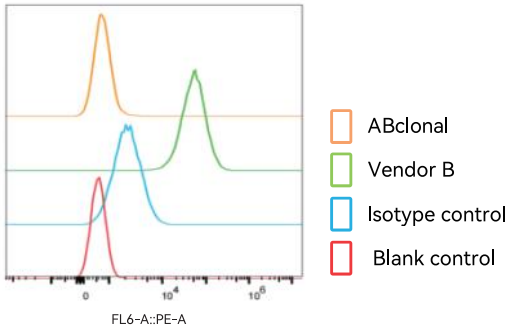


CHO Claudin18.2 stable cell line

Results: Antibodies obtained from Claudin 18.2 stable cell line immunization showed significantly improved signal transition in flow cytometry under the same experimental conditions compared to competitors.

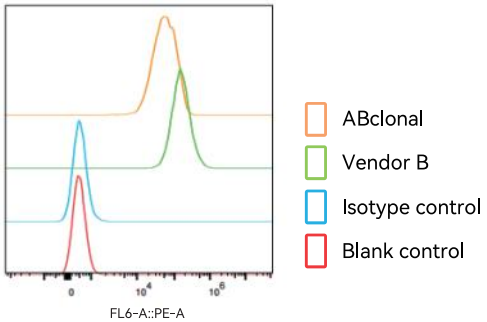
High specificity ensures reliable results

Human CD59-Cat.No.A23365



U937 cells (negative control)

Human CD59-Cat.No.A23365

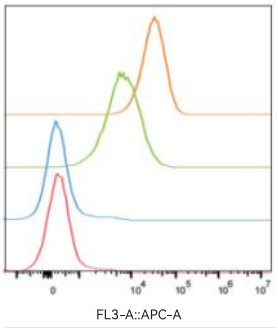


Jurkat cells

The results showed that under the same experimental conditions, ABclonal antibody exhibited no significant signal transition in the negative control sample (U937 cells) but displayed a noticeable signal transition in the positive sample (Jurkat cells), indicating higher antibody specificity compared to competitors.

Low working concentration leads to cost savings

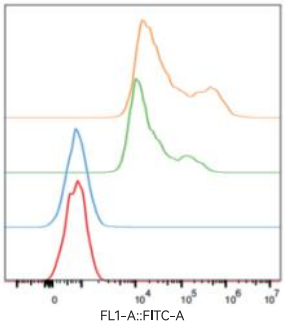
Mouse CD25-Cat.No.A23808



ABclonal (0.5 μg/mL)
Vendor B (2.5 μg/mL)
Isotype control
Blank control

CTLL-2 cells

Human CD62L-Cat.No.A23020



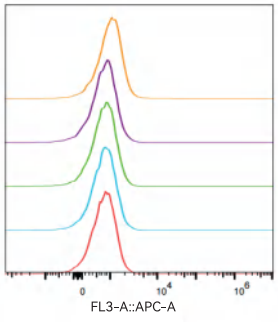
ABclonal (2 μg/mL)
Vendor B (10 μg/mL)
Isotype control
Blank control

MOLT-4 cells

The results showed that under the same experimental conditions, ABclonal antibody exhibited significant signal transition, even with lower usage compared to competitor antibodies.

Selection of high-performance clones for production

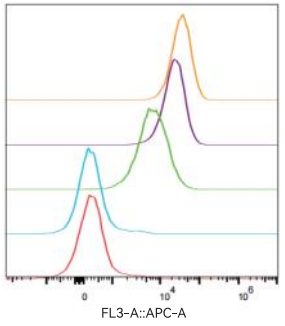
Mouse CD25-Cat.No.A23808



ABclonal (Clone ARC54525-ABf647)
ABclonal (Clone ARC54526-ABf647)
Vendor B
Isotype control
Blank control

C2C12 cells (negative control)

Mouse CD25-Cat.No.A23808

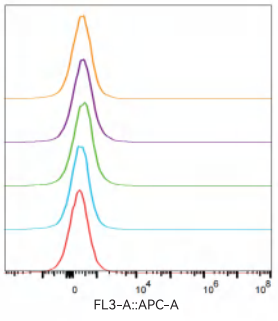


ABclonal (Clone ARC54525-ABf647)
ABclonal (Clone ARC54526-ABf647)
Vendor B
Isotype control
Blank control

CTLL-2 cells

Results showed that under the same experimental conditions, the clone ARC54525-ABf647 from ABclonal outperformed the clone ARC54526-ABf647 and competitors, leading to its selection for production.

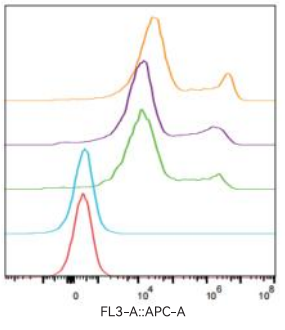
Mouse CD117-Cat.No.A23582



ABclonal (Clone ARC60551-ABf647)
ABclonal (Clone ARC60546-ABf647)
Vendor B
Isotype control
Blank control

293F cells (negative control)

Mouse CD117-Cat.No.A23582



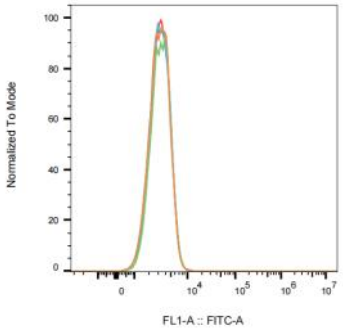
ABclonal (Clone ARC60551-ABf647)
ABclonal (Clone ARC60546-ABf647)
Vendor B
Isotype control
Blank control

293F cells (transfection)

Results showed that under the same experimental conditions, the clone ARC60551-ABf647 from ABclonal outperformed the clone ARC60546-ABf647 and competitors, leading to its selection for production.

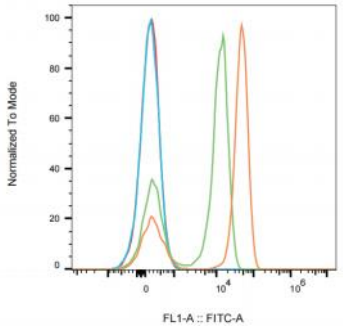
Optimal host selection for targeting human and mouse proteins

Human CD3-Cat.No.A24060

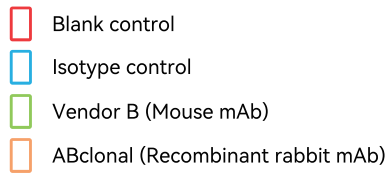


PC-3 cells (negative control)

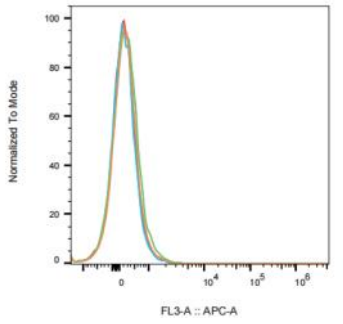
Human CD3-Cat.No.A24060



PBMC

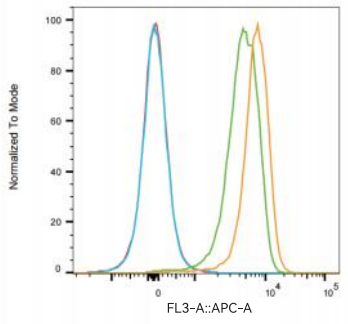


Human CD79b-Cat.No.A23802

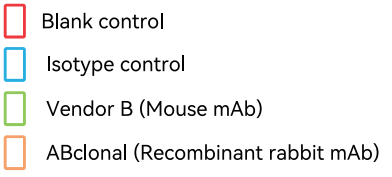


HAP1 cells (negative control)

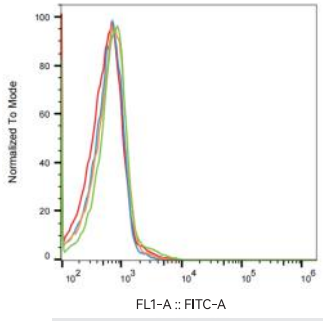
Human CD79b-Cat.No.A23802



Daudi cells

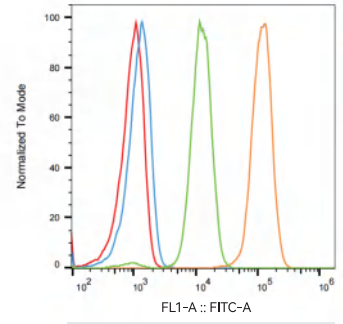


Human LAMP2-Cat.No.A22216

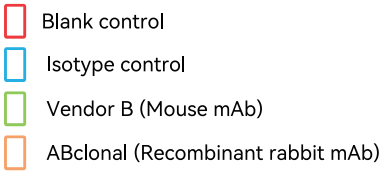


HeLa cells (knockout)

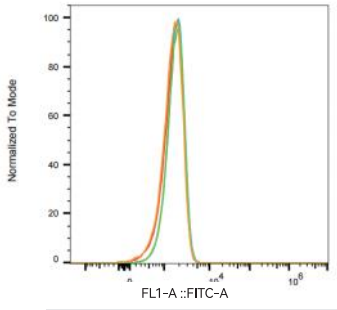
Human LAMP2-Cat.No.A22216



HeLa cells

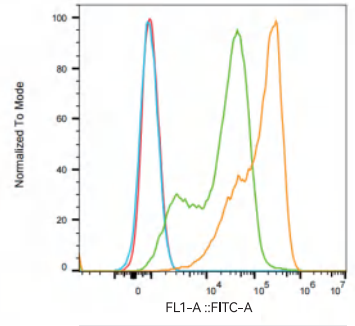


Mouse CD102-Cat.No.A24241

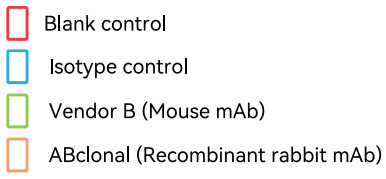


C2C12 cells (low expression)

Mouse CD102-Cat.No.A24241



bEnd.3 cells

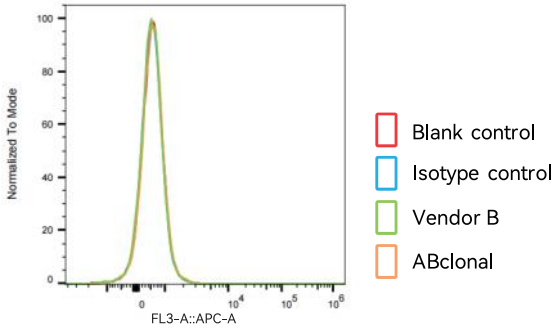


1.2.2 Comprehensive Validation and Rigorous Data

A wide range of samples, such as cell lines, PBMCs, and splenocytes, are carefully chosen based on the specific target characteristics, enabling comprehensive antibody validation following screening and conjugation. Each antibody undergoes meticulous optimization, including titration and buffer formulation optimization, to ensure stable results and a seamless user experience. Well-established standard operating procedures and mature production processes ensure excellent product stability, while stringent quality control measures are implemented across multiple batches to ensure exceptional consistency between them.

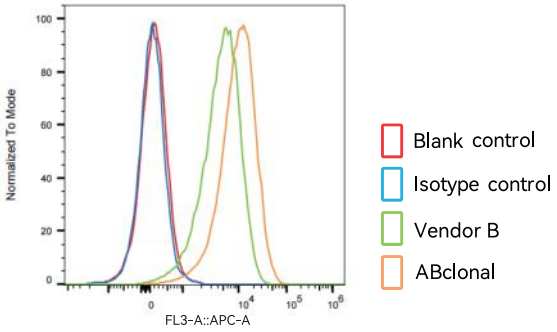
A broad range of sample types

Human/Monkey CD8a-Cat.No.A23346



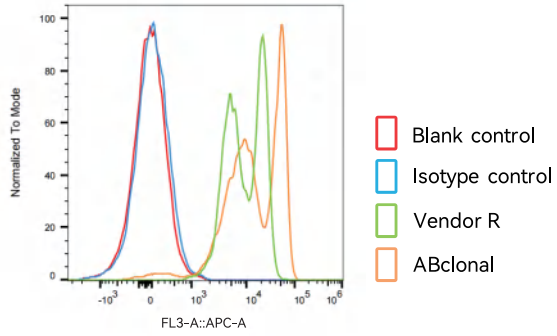
293F cells (negative control)

Human/Monkey CD8a-Cat.No.A23346



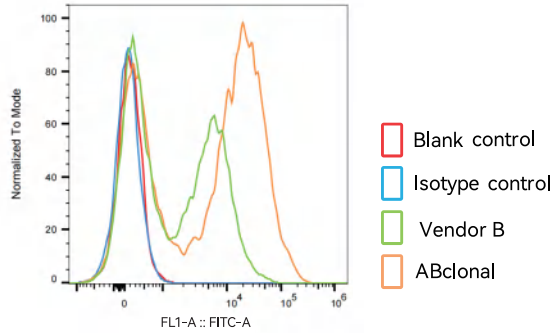
MOLT-4 cells

Human CD85a-Cat.No.A24712



Human PBMC

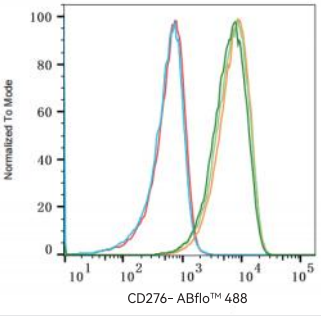
Mouse IgM-Cat.No.A23114



C57BL/6 mouse Splenocytes

Titration improves signal-to-noise ratio

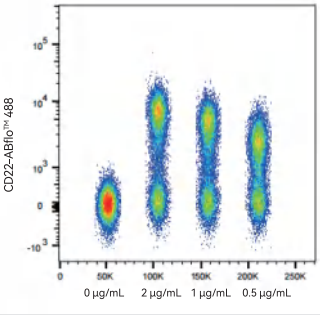
Human/Mouse CD276-Cat.No.A22156



Sample: PC-3 cells
Recommended antibody concentrations:0.5 μg/mL

0.5 μg/mL
1 μg/mL
2 μg/mL

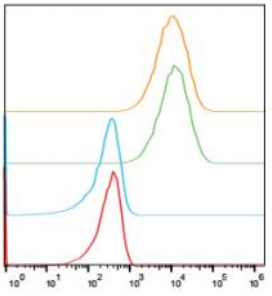
Mouse CD22-Cat.No.A24627



Sample: C57BL/6 mouse Splenocytes
Recommended antibody concentrations: 2 μg/mL

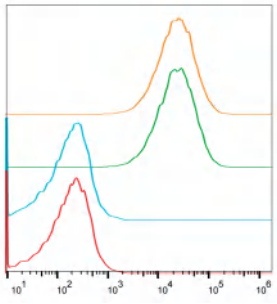
High inter-batch consistency

Human CD45RA-Cat.No.A22569



Jurkat cells

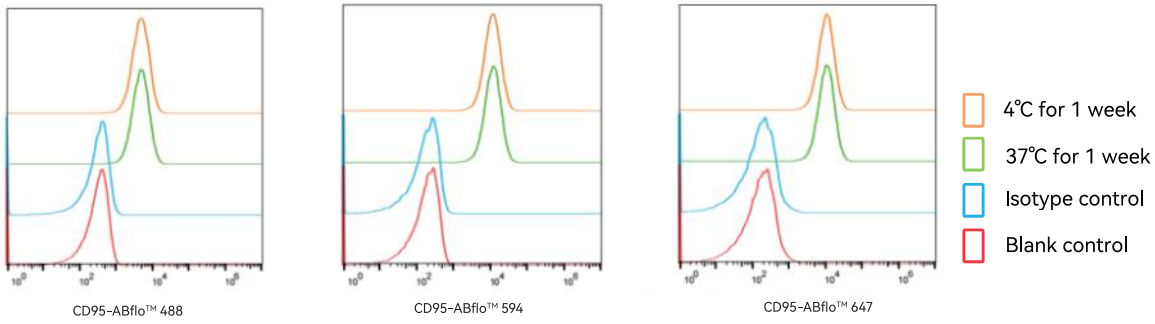
Human CD45RA-Cat.No.A22571



Jurkat cells

Good stability

Mouse CD95-Cat.No.A23810 | Mouse CD95-Cat.No.A23812 | Mouse CD95-Cat.No.A23811



A20 cells

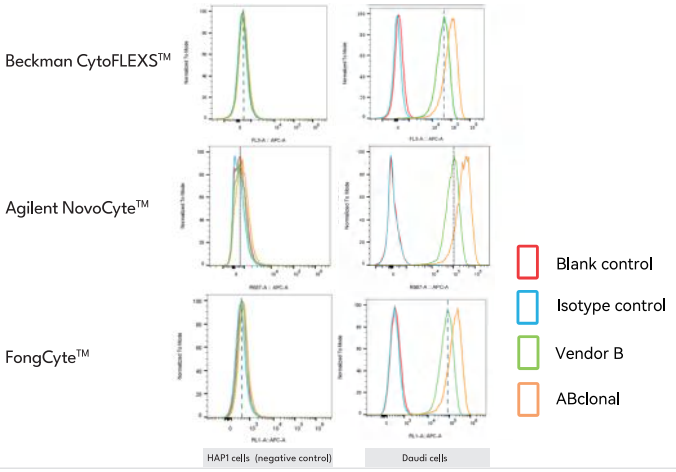
1.2.3 Excellent Compatibility and High Reproducibility

Through the use of mainstream flow cytometry analysis and various compatible reagents, the results exhibit high consistency, reliable cross-platform data, and good transferability.

Equipment compatibility validation

Targe: Human CD79b
Fluorophore: ABflo™ 647 (ABclonal Cat.No.A23802)
APC (Competitor antibody)

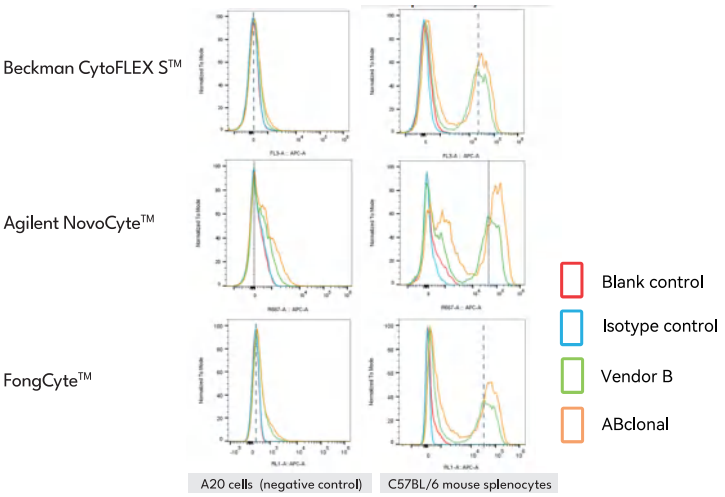
The results demonstrate that when utilizing ABflo™ 647-conjugated antibodies targeting human CD79b in comparison to competitor antibodies conjugated with APC, under identical experimental conditions, detection was conducted using the APC channel on various flow cytometers, including Beckman cytoflex™ S, Agilent NovoCyte™, and FongCyte™. ABclonal flow cytometry antibodies consistently exhibited comparable signal transitions across all three devices, outperforming the competitors with significantly enhanced signal transitions.



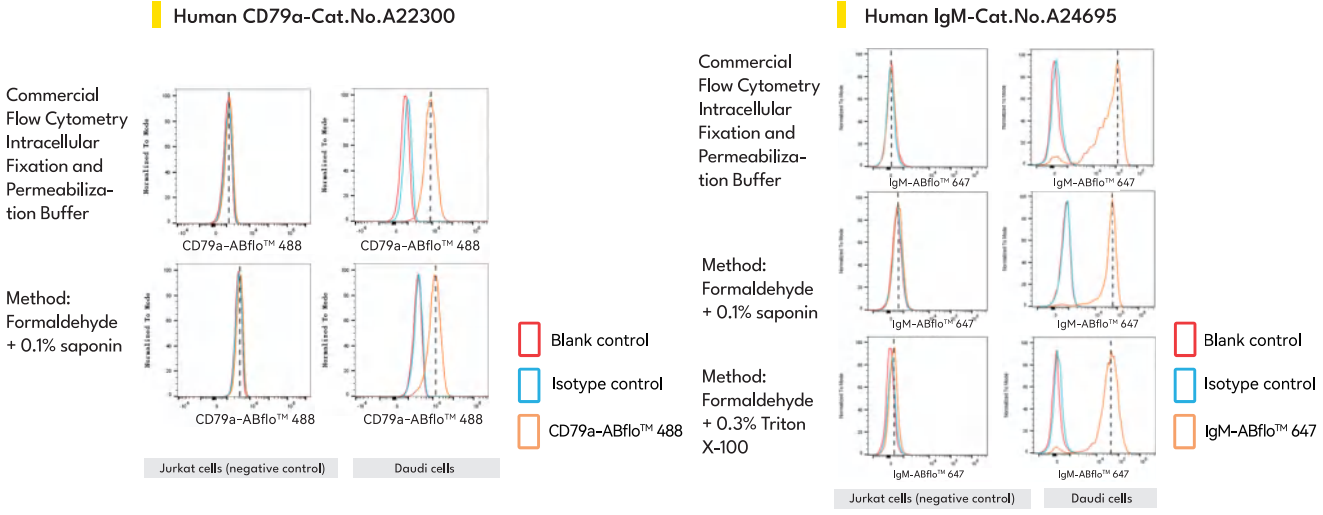
Equipment compatibility validation

Targe: Mouse CD5
Fluorophore: ABflo® 647
(ABclonal Cat.No.A23972)
APC (Competitor antibody)

The results demonstrate that when employing ABflo™ 647-conjugated antibodies targeting mouse CD5 alongside competitor antibodies conjugated with APC, under identical experimental conditions, detection was carried out utilizing the APC channel on various flow cytometers, including Beckman cytoflex™ S, Agilent NovoCyte™, and FongCyte™. ABclonal flow cytometry antibodies consistently exhibited comparable signal transitions across all three devices, exhibiting slightly superior signal transitions compared to the competitors.



Reagent compatibility validation



1.2.4 Stable Supply Chain, Guaranteed Availability

Through our stable supply chain, we ensure timely, convenient, fast, secure, and reliable delivery. Our supply system offers flexibility, enabling customized production and labeling of our existing products to meet specific requirements.

2. ABflo™ Series Fluorescent Dyes

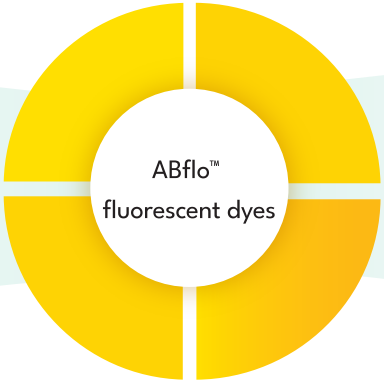
2.1 Features of ABflo™ Fluorescent Dyes

High fluorescence intensity

The ABflo™ series dyes exhibit excellent fluorescence intensity, allowing for better detection and discrimination of weakly expressing cell populations.

High stability

These dyes have minimal decay in luminescence stability, enabling long-term acquisition and analysis of high-quality fluorescence data.



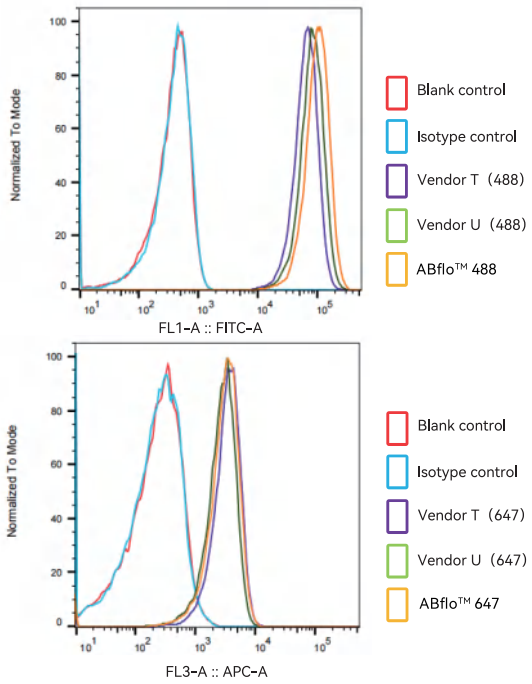
Wide pH tolerance range

The dyes exhibit stable fluorescence across a broad pH range, maintaining stability within a wide pH range.

Broad instrument compatibility

ABflo™ dyes have good spectral compatibility with common flow cytometry analysis and sorting devices, ensuring reliable data.

Flow cytometry experiments were conducted under same conditions using ABclonal's recombinant rabbit monoclonal antibodies targeting the same antigen, sourced from the same batch. A comparative analysis was performed between ABflo™ dyes and other dyes with similar spectra for labeling. The results showed that the ABflo™ series fluorescent dyes exhibited superior or comparable brightness levels when compared to widely used fluorescent dyes.



- Sample: U266 Cells
- Unconjugated Antibody: Rabbit anti-Human CD48 mAb
- Results: ABflo™ 488 >Vendor U (488)>Vendor T (488)

- Sample: Raji Cells
- Unconjugated Antibody: Rabbit anti-Human CD40 mAb
- Results: ABflo™ 647≈Vendor T (647)> Vendor U (647)

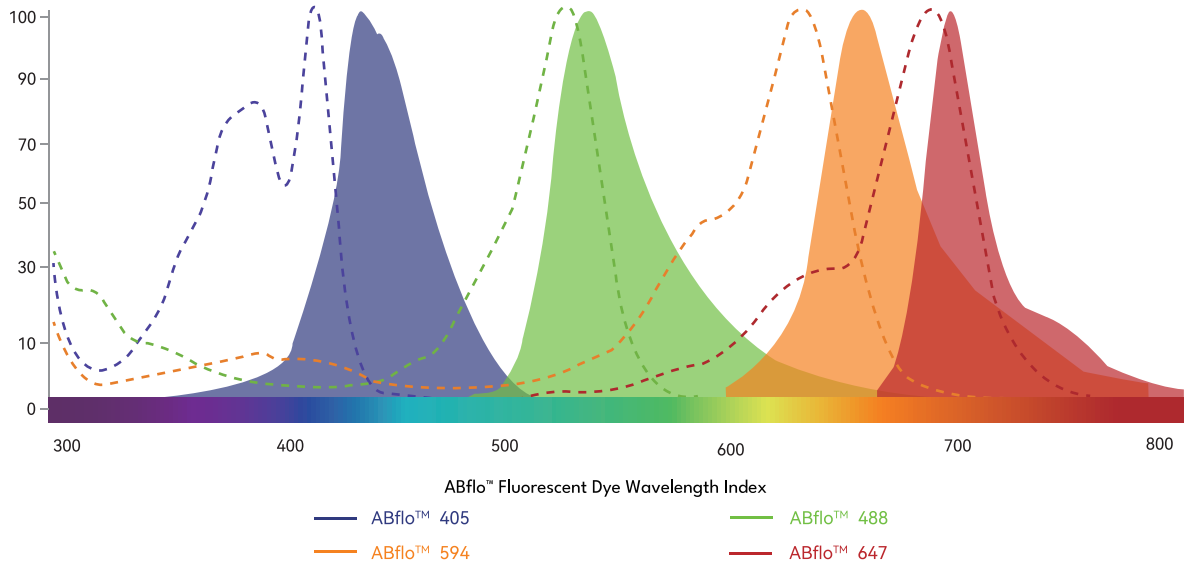
2.2 Common Fluorescent Dyes and Selection

Dye Name	Maximum Excitation Wavelength (nm)	Maximum Emission Wavelength (nm)	Fluorescent Dyes in the Same Channel (Partial Listing)
ABflo™ 405	403	427	Pacific Blue, Alexa Fluor™ 405, eFluor450
ABflo™ 488	491	516	FITC, BB515, Alexa Fluor™ 488
FITC	491	516	ABflo™ 488, Alexa Fluor™ 488, BB515
ABflo™ 594	588	604	Alexa Fluor™ 594, ECD
PE	565	574	-
PerCP	482	678	PerCP-Cy5.5, BB700
PerCP-Cy5.5	482	695	PerCP, BB700
ABflo™ 647	648	664	APC, Alexa Fluor™ 647, eFluor660
APC	650	660	ABflo™ 647, Alexa Fluor™ 647, eFluor660
ABflo™ 700	690	713	Alexa Fluor™ 700, APC-Alexa Fluor™ 700
PE-Cy5	565	666	PE-Alexa Fluor™647
PE-Cy7	565	778	PE-Vio™770
APC-Cy7	651	779	APC-H7

Intensity of Common Fluorescent Dyes: PE-Cy5>PE>PE-Cy7>ABflo™ 647>APC>APC-Cy7>ABflo™ 594>ABflo™ 700>ABflo™ 488> PerCP-Cy5.5>ABflo™ 405>FITC>PerCP

Tips for Fluorescent Dye Selection:

- Instrument Configuration: Before performing flow cytometry staining, it is essential to understand your instrument's configuration (lasers, filters).
- Dye Brightness: Different dyes exhibit varying fluorescence intensities. A comparison can be made using stain index, where higher stain index correspond to brighter fluorescence.
- Antigen Expression and Dye Pairing: Pair weakly expressed antigens with brighter fluorescent dyes, while strongly expressed antigens can be paired with less intense fluorescent dyes.
- Spectral Overlap: When combining different channel fluorescent dyes, select dyes with minimal overlap in emission spectra.
- Fluorescent Interference: Use fluorescent dyes that do not interfere with each other to label different cell populations and analyze them separately by gating.
- Cell Viability Detection Dyes: Flow cytometry multicolor panels should include cell viability detection dyes to exclude dead cells and cell debris.



3. Common Cell Phenotyping Markers

3.1 Leukocyte Common Antigen

CD45, also known as Leukocyte Common Antigen (L-CA), is a widely expressed membrane glycoprotein found on all hematopoietic cells and their various differentiated subpopulations, except for platelets and red blood cells. It is a commonly used marker for hematopoietic cells.

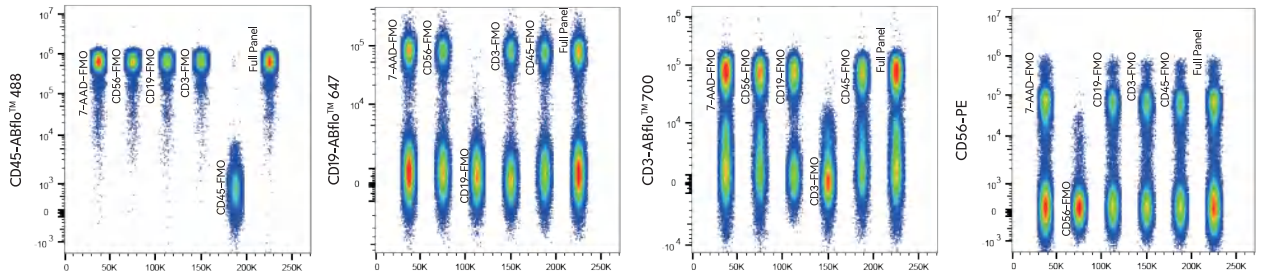
Case Study: Common Immunocyte Phenotyping (T, B, NK cells)

(1) Panel Design

Fluorescent Dyes	7-AAD-FMO	PE-FMO	ABflo™ 647-FMO	ABflo™700-FMO	ABflo™ 488-FMO	Full Panel	Species	Cat.No.	Sample
ABflo™ 488	CD45-ABflo™ 488	CD45-ABflo™ 488	CD45-ABflo™ 488	CD45-ABflo™ 488	-	CD45-ABflo™ 488	Human	A22494	Human PBMC
ABflo™ 647	CD19-ABflo™ 647	CD19-ABflo™ 647	-	CD19-ABflo™ 647	CD19-ABflo™ 647	CD19-ABflo™ 647	Human	A23009	
ABflo™700	CD3-ABflo™700	CD3-ABflo™700	CD3-ABflo™700	-	CD3-ABflo™700	CD3-ABflo™700	Human	A24943	
PE	CD56-PE	-	CD56-PE	CD56-PE	CD56-PE	CD56-PE	Human	A22826	
7-AAD	-	7-AAD	7-AAD	7-AAD	7-AAD	7-AAD	Human	-	

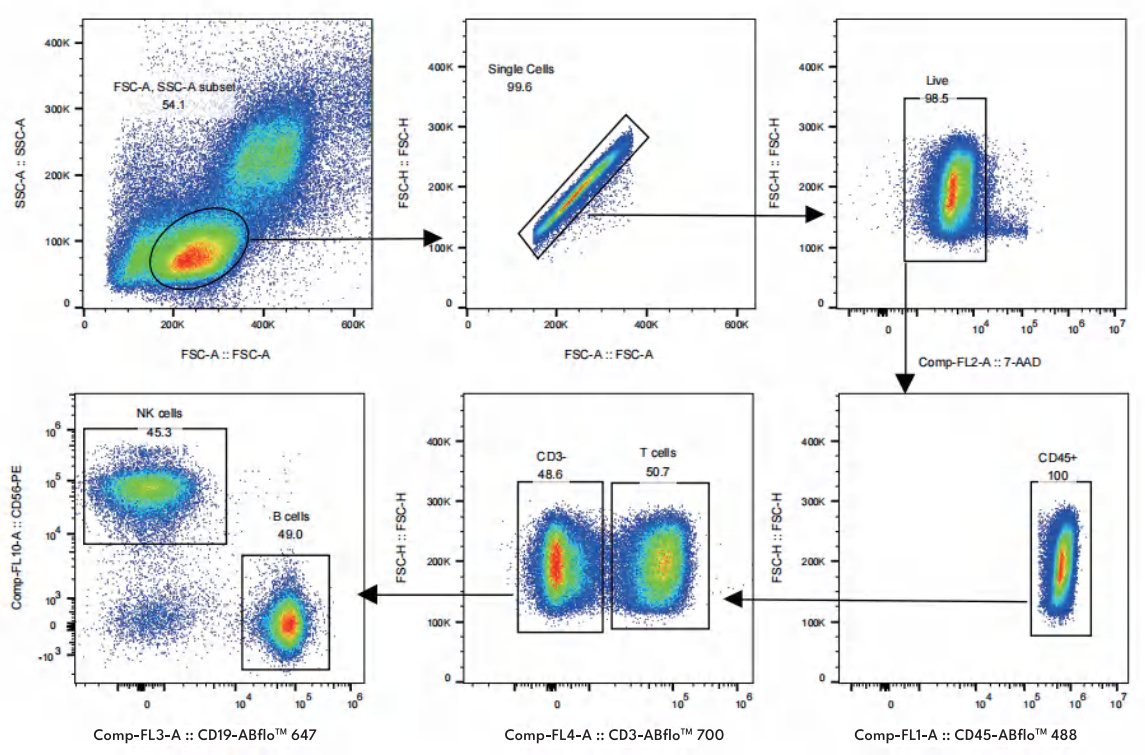
(2) Multicolor Manual Gating Analysis

FMO Controls



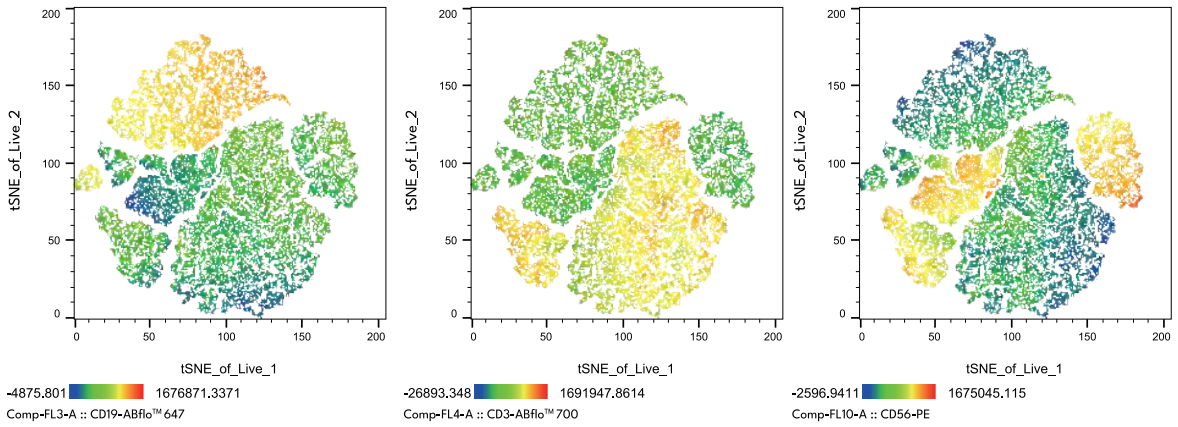
Results: FMO controls for 7-AAD, CD56-PE, CD19-ABflo™ 647, CD3-ABflo™ 700, and CD45-ABflo™488 demonstrated distinct separation between the positive and negative populations, enabling precise differentiation of cell populations. The remaining fluorescent dyes employed in the experimental panel exhibited minimal interference with the observed signal in the channels associated with the target fluorescent dyes.

Full Stained Sample



Results: Multicolor staining with CD45, CD19, CD3, CD56, and 7-AAD resulted in clear separation of $CD3^+$ T cells, $CD3^-$ $CD19^+$ B cells, and $CD3^-$ $CD19^-$ $CD56^+$ NK cells.

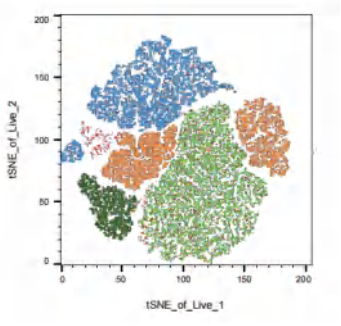
(3) tSNE Dimensionality Reduction Analysis of Signal Distribution for Each Marker



Results: In this plot, colors ranging from red to blue represent the expression of the corresponding marker. The signal distribution plots for CD3, CD19, and CD56 were consistent with the clustering of T cells, B cells, and NK cells, respectively.

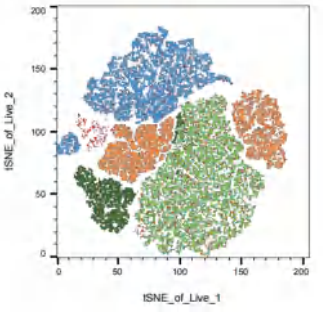
(4) Comparison of Manual Gating and Automatic tSNE Analysis

Manual Gating



Subset Name	Count
CD3⁺ CD56⁺ NKT cell	6770
CD3⁺ CD56⁻ T cell	39515
NK cells	20097
B cells	21093
Live	92771

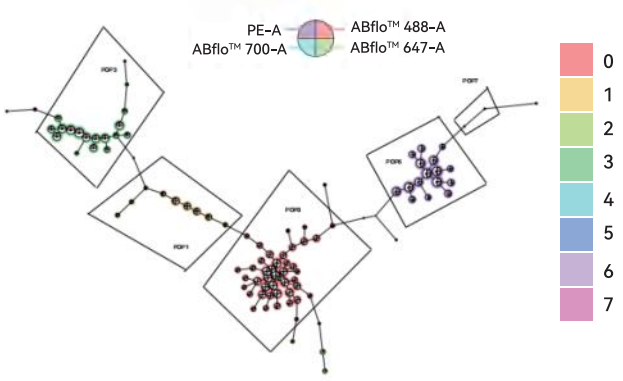
Automatic tSNE Analysis



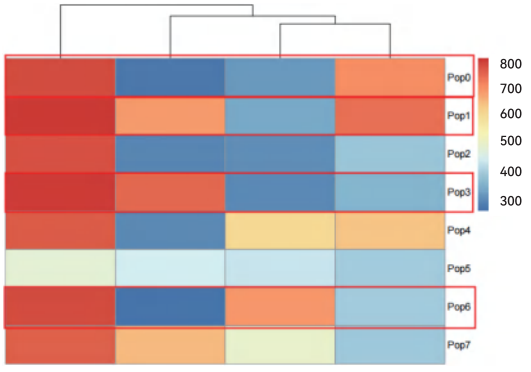
Subset Name	Count
FlowSOM.Live.Pop1.NKT cell	7647
FlowSOM.Live.Pop0.T cell	39203
FlowSOM.Live.Pop3.NK cell	20069
FlowSOM.Live.Pop6.B cell	21452
Live	92771

Results: In this plot, live cell populations gated from lymphocytes are depicted in red, CD19⁺ B cells (CD45⁺ CD3⁻ CD19⁺) in blue, NK cells (CD45⁺ CD3⁻ CD56⁺) in orange, and CD3⁺ CD56⁻ T cells (CD3⁺ CD56⁻) in green. CD3⁺ CD56⁺ NKT cells are represented by the dark green color. Both manual gating and FlowSOM automated analysis consistently demonstrated clustering and accurate cell counts.

(5) FlowSOM Clustering Analysis



Results: The plot showcases the clustering results obtained using FlowSOM, visualized through a minimum spanning tree. Cells belonging to the same cluster are denoted by the same background color, while the size of the bubbles represents the relative sizes of the respective clusters. The sector plot indicates the expression of the corresponding marker, with larger sectors indicating higher expression and vice versa.



Results: The plot depicts a heatmap generated by FlowSOM, where varying colors from red to blue indicate the expression strength of the corresponding markers. Specifically, POP0 represents the $CD3^+$ $CD56^-$ T cell subset ($CD45^+$ $CD3^+$ $CD19^-$ $CD56^-$), POP1 represents the $CD3^+$ $CD56^+$ NKT cell subset ($CD45^+$ $CD3^+$ $CD19^-$ $CD56^+$), POP3 represents the NK cell subset ($CD45^+$ $CD3^-$ $CD19^-$ $CD56^+$), and POP6 represents the $CD19^+$ B cell subset ($CD45^+$ $CD3^-$ $CD19^+$ $CD56^-$). The heatmap provides a comprehensive overview of marker expression patterns within these distinct cell subsets.

Flow cytometry antibodies related to Leukocyte Common Antigen

Target	Fluorophore	Reactivity	Cat.No.
CD45	ABflo™488	Human	A22494
	ABflo™647	Human	A22495

Target	Fluorophore	Reactivity	Cat.No.
CD45	ABflo™488	Mouse	A23707
	ABflo™594	Mouse	A23709
	ABflo™647	Mouse	A23708

3.2 T Lymphocytes

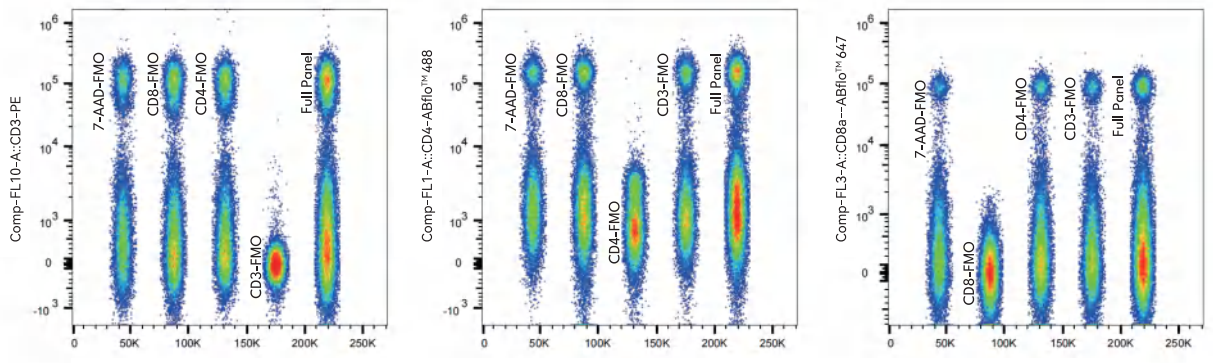
T lymphocytes, originating from hematopoietic stem cells within the bone marrow, undergo a process of differentiation and maturation within the thymus. Subsequently, they disperse throughout the immune organs and tissues of the body via the lymphatic and circulatory systems, assuming crucial roles in immune function. T cells play a pivotal role in cell-mediated immune responses, and their functional abnormalities are closely linked to various disease states. Throughout distinct stages of differentiation and maturation, T cells exhibit the expression of diverse surface membrane proteins, including CD4 (a marker for helper T cells), CD8 (a marker for cytotoxic T cells), and CD3 (a pan-T cell marker), among others. Additionally, they possess the vital functions of antigen recognition, mediating specific immune responses, and regulating the immune system overall.

Case Study: T Cell Immunophenotyping

(1) Panel Design

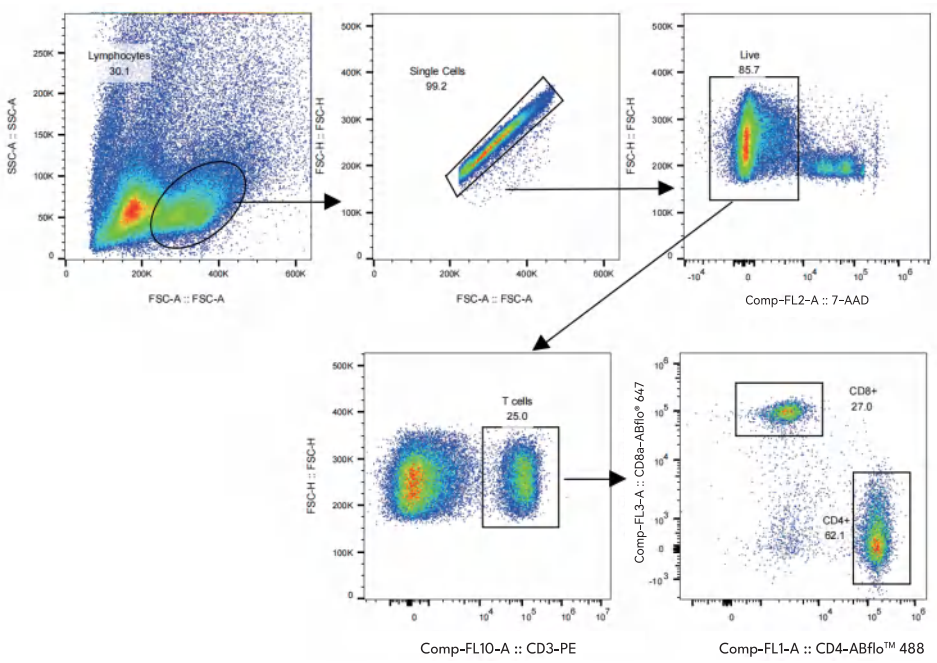
Fluorescent Dyes	7-AAD-FMO	ABflo™647-FMO	ABflo™488-FMO	PE-FMO	Full Panel	Species	Cat.No.	Sample
ABflo™ 488	CD4-ABflo™ 488	CD4-ABflo™ 488	-	CD4-ABflo™ 488	CD4-ABflo™ 488	Mouse	A24907	Mouse Spleen
ABflo™ 647	CD8a-ABflo™ 647	-	CD8a-ABflo™ 647	CD8a-ABflo™ 647	CD8a-ABflo™ 647	Mouse	A24942	
PE	CD3-PE	CD3-PE	CD3-PE	-	CD3-PE	Mouse	A23321	
7-AAD	-	7-AAD	7-AAD	7-AAD	7-AAD	Mouse	-	

(2) FMO Controls



Results: Staining with 7-AAD-FMO, CD8a-ABflo™ 647-FMO, CD4-ABflo™ 488-FMO, and CD3-PE-FMO showed clear separation between positive and negative cell populations.

(3) Full Stained Sample



Results: Multicolor staining with CD3, CD4, CD8, and 7-AAD resulted in clear separation of $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells.

Flow cytometry antibodies related to T lymphocyte phenotyping

Target	Fluorophore	Reactivity	Cat.No.
CD2	ABflo™ 488	Human	A23116
	ABflo™ 647	Human	A23117
	ABflo™ 488	Mouse	A23704
	ABflo™ 647	Mouse	A23705
CD3	FITC	Human	A22794
	PE	Human	A22795
	PE-Cy7	Human	A22798
	PerCP	Human	A22796
	PerCP-Cy5.5	Human	A22797

Target	Fluorophore	Reactivity	Cat.No.
CD4	ABflo™ 647	Dog	A22688
	ABflo™ 488	Pig	A22774
	ABflo™ 647	Pig	A22775
CD7	ABflo™ 488	Human	A22193
	ABflo™ 647	Human	A22194
CD8	ABflo™ 488	Human,Monkey	A23345
	ABflo™ 647	Human,Monkey	A23346
	ABflo™ 488	Mouse	A23903
	ABflo™ 647	Mouse	A23904

Target	Fluorophore	Reactivity	Cat.No.
CD3	FITC	Mouse	A23322
	ABflo™ 488	Cat	A22490
	ABflo™ 647	Cat	A22491
	ABflo™ 594	Dog	A23352
	ABflo™ 647	Dog	A23353
CD4	ABflo™ 488	Human,Monkey	A22773
	ABflo™ 488	Mouse	A24720
	ABflo™ 594	Mouse	A24722
	ABflo™ 647	Mouse	A24721
	ABflo™ 488	Dog	A22687

Target	Fluorophore	Reactivity	Cat.No.
CD25	ABflo™ 594	Mouse	A23809
	ABflo™ 647	Mouse	A23808
CD28	ABflo™ 488	Human	A23405
	ABflo™ 647	Human	A23406
CD62L	ABflo™ 488	Human	A23020
	PE	Human	A24249
CD127	PE	Human	A22842
	APC	Human	A22843
	ABflo™ 488	Mouse	A24788
TIGIT	ABflo™ 647	Human	A23354

3.3 B Lymphocytes

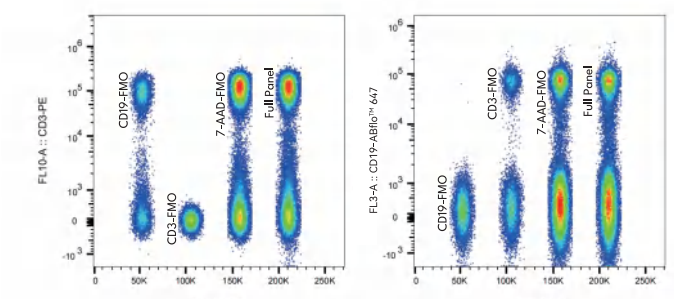
B lymphocytes, originating from hematopoietic stem cells within the bone marrow, undergo maturation within the bone marrow itself before migrating to lymph nodes and the spleen. These cells possess the ability to further differentiate into memory B cells or plasma cells. B cells serve as the principal mediators of humoral immunity, generating immune responses through the secretion of antibodies. Throughout the development and maturation of B cells, they exhibit the expression of diverse markers, including CD19 and CD20 as pan-B cell markers, as well as CD5 and CD21 as markers for Breg cells, among others. By employing specific antibodies, it becomes possible to distinguish specific subsets of B cells within heterogeneous populations of immune cells.

Case Study: B Cell Phenotyping

(1) Panel Design

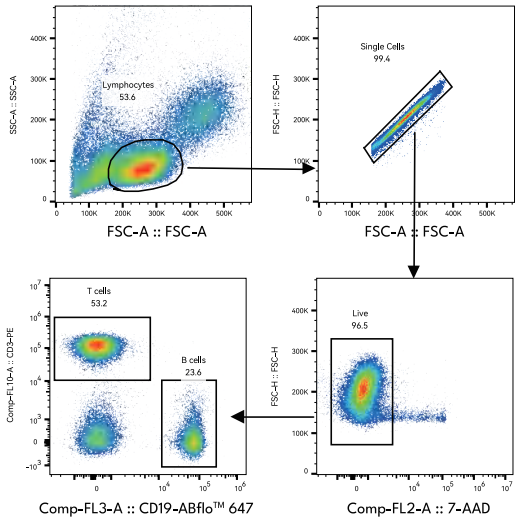
Fluorescent Dyes	7-AAD-FMO	ABflo™ 647-FMO	PE-FMO	Full Panel	Species	Cat.No.	Sample
ABflo™ 647	CD19-ABflo™ 647	-	CD19-ABflo™ 647	CD19-ABflo™ 647	Human	A23009	Human PBMC
PE	CD3-PE	CD3-PE	-	CD3-PE	Human	A22795	
7-AAD	-	7-AAD	7-AAD	7-AAD	Human	-	

(2) FMO Controls



Results: Staining with CD19-ABflo™647-FMO, CD3-PE-FMO, and 7-AAD-FMO showed clear separation between positive and negative cell populations.

(3) Full Stained Sample



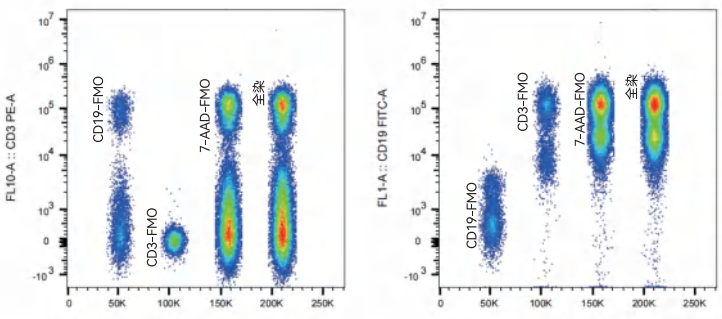
Results: Multicolor staining with CD3, CD19, and 7-AAD resulted in clear separation of $CD3^+$ T cells and $CD3^-CD19^+$ B cells.

Case Study: B Cell Phenotyping

(1) Panel Design

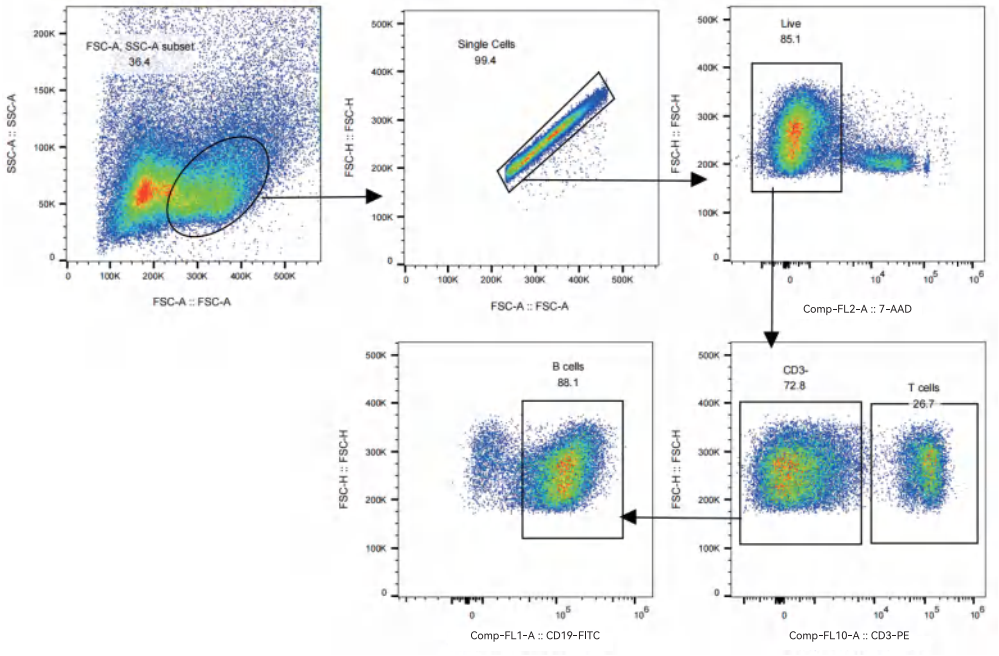
Fluorescent Dyes	7-AAD-FMO	FITC-FMO	PE-FMO	Full Panel	Species	Cat.No.	Sample
FITC	CD19-FITC	-	CD19-FITC	CD19-FITC	Mouse	A23330	Mouse Spleen
PE	CD3-PE	CD3-PE	-	CD3-PE	Mouse	A23321	
7-AAD	-	7-AAD	7-AAD	7-AAD	Mouse	-	

(2) FMO Controls



Results: Staining with CD19-FITC-FMO, CD3-PE-FMO, and 7-AAD-FMO showed clear separation between positive and negative cell populations.

(3) Full Stained Sample



Results: Multicolor staining with CD3, CD19, and 7-AAD resulted in clear separation between positive and negative cell populations.

Flow cytometry antibodies related to B lymphocyte phenotyping

Target	Fluorophore	Reactivity	Cat.No.
CD5	ABflo™488	Human	A22185
	ABflo™647	Human	A22186
	ABflo™488	Mouse	A23971
	ABflo™647	Mouse	A23972
CD19	ABflo™488	Human,Monkey	A23008
	ABflo™647	Human,Monkey	A23009
	FITC	Human	A22815
	PE	Human	A22816
	PE-Cy7	Human	A22819
	PerCP-Cy5.5	Human	A22818
	APC	Human	A22820
	APC-Cy7	Human	A22821
	FITC	Mouse	A23330
	PE	Mouse	A23331
	ABflo™488	Cat	A23100
	ABflo™647	Dog	A23347
	PE	Dog	A23592
	ABflo™647	Pig	A23396
CD20	ABflo™488	Human,Monkey	A22152
	ABflo™647	Human,Monkey	A22153
	PE	Human	A24420
CD21	ABflo™488	Human	A22643
	ABflo™594	Human	A23367
	ABflo™647	Human	A22644

Target	Fluorophore	Reactivity	Cat.No.
CD27	ABflo™488	Human	A22063
	ABflo™647	Human	A22064
	ABflo™488	Cat	A23171
	ABflo™647	Cat	A23172
	ABflo™488	Dog	A22575
	ABflo™647	Dog	A22576
	ABflo™488	Pig	A23101
	ABflo™647	Pig	A23102
CD38	ABflo™488	Human	A23747
	ABflo™647	Human	A24365
	ABflo™488	Mouse	A24622
	ABflo™647	Mouse	A24623
CD40	ABflo™488	Human	A22641
	ABflo™647	Human	A22642
IgA	ABflo™488	Cynomolgus monkey	A23584
	ABflo™647	Cynomolgus monkey	A23585
IgD	ABflo™488	Human	A23112
	ABflo™647	Human	A23113
IgE	ABflo™488	Human	A23696
	ABflo™647	Human	A23697
IgG	ABflo™488	Human	A22504
	ABflo™647	Human	A22505
IgM	ABflo™488	Mouse	A23114
	ABflo™647	Mouse	A23115

3.4 NK Cells

Natural killer cells (NK cells) originate from lymphoid progenitor cells within the bone marrow and are classified as innate lymphoid cells. They perform a crucial role in the frontline defense against invading pathogens and tumors, operating independently of specific antigen recognition or co-stimulation. The activity of NK cells serves as a valuable indicator of the body's immune response against tumors and viral infections. Commonly employed phenotypic markers for human NK cells include CD56 and CD16.

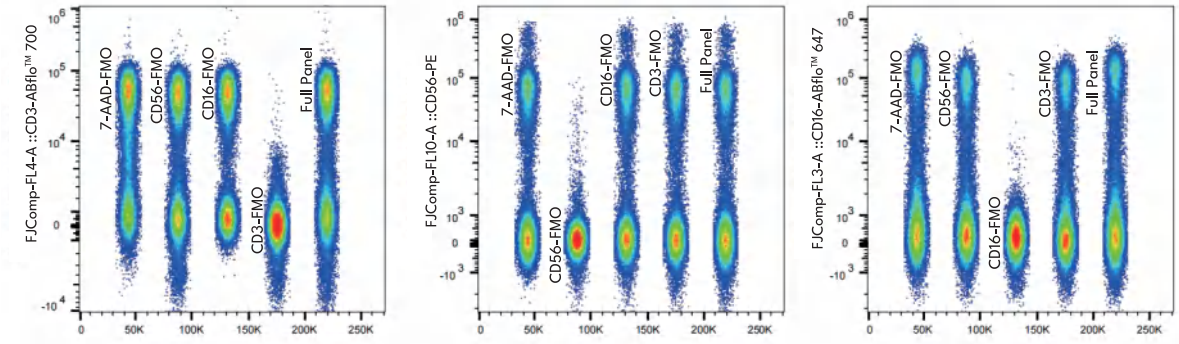
Case Study: NK Cell Phenotyping

(1) Panel Design

Fluorescent Dyes	7-AAD-FMO	PE-FMO	ABflo™ 647-FMO	ABflo™ 700-FMO	Full Panel	Species	Cat.No.	Sample
ABflo™ 647	CD16-ABflo™ 647	CD16-ABflo™ 647	-	CD16-ABflo™ 647	CD16-ABflo™ 647	Human	A23400	Human PBMC
ABflo™ 700	CD3-ABflo™ 700	CD3-ABflo™ 700	CD16-ABflo™ 700	-	CD3-ABflo™ 700	Human	A24943	
PE	CD56-PE	-	CD56-PE	CD56-PE	CD56-PE	Human	A22826	
7-AAD	-	7-AAD	7-AAD	7-AAD	7-AAD	Human	-	

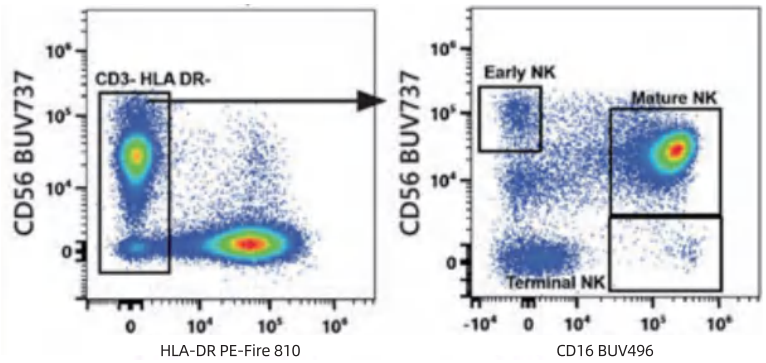
(2) Multicolor Manual Gating Analysis

FMO Controls



Results: Staining with 7-AAD-FMO, CD56-PE-FMO, CD16-ABflo™ 647, and CD3-ABflo™ 700 showed clear separation between positive and negative cell populations.

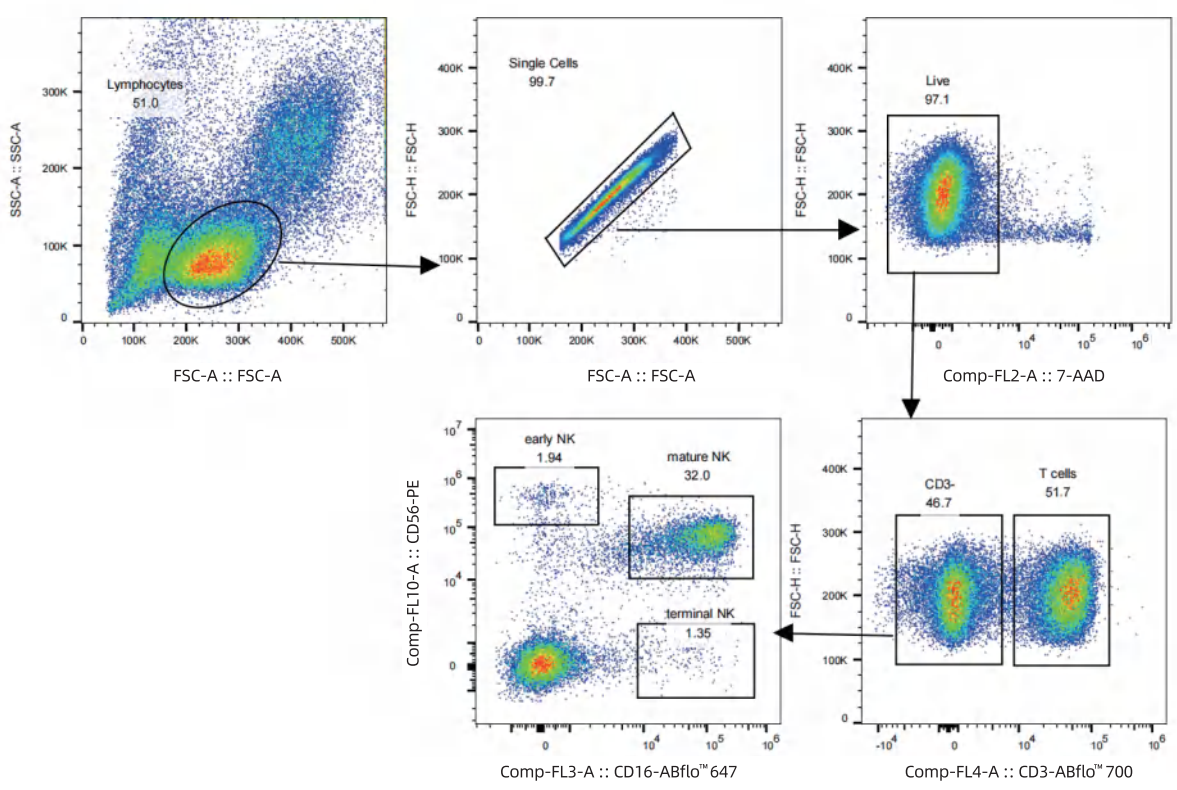
NK Cell Subsets



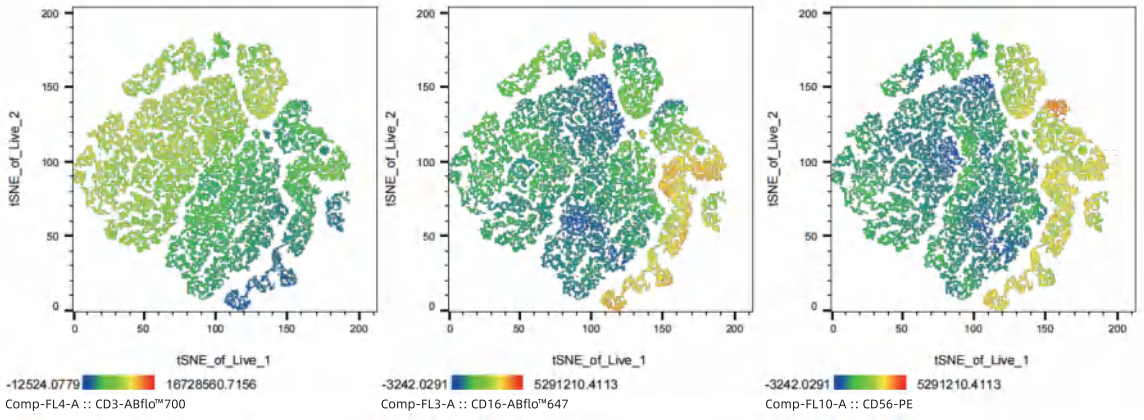
Source: DOI: 10.1002/cyto.a.24213

Results: Multicolor staining with CD3, CD16, CD56, and 7-AAD resulted in clear separation of $CD16^+$ $CD56^+$ cells, $CD16^+$ $CD56^-$ cells, and $CD16^-$ $CD56^+$ cells, consistent with the reference literature.

Full Stained Sample



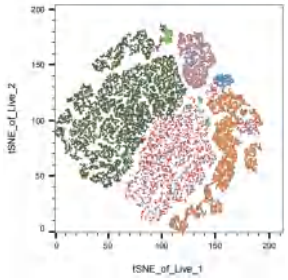
(3) tSNE Dimensionality Reduction Analysis of Signal Distribution for Each Marker



Results: The plot exhibits a color gradient ranging from red to blue, reflecting the expression of the respective markers. Notably, the signal value distribution of CD3, CD16, and CD56 aligns consistently with the clustering patterns observed for T cells and NK cells.

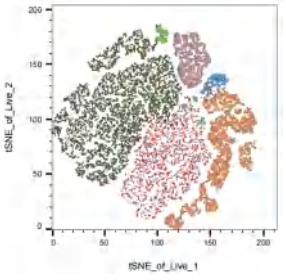
(4) Comparison of Manual Gating and Automatic tSNE Analysis

Manual Gating



	Subset Name	Count
	$CD3^+$ $CD56^+$ NKT cell	3508
	$CD3^+$ $CD56^-$ T cell	23760
	terminal NK	516
	mature NK	7012
	early NK	520
	Live	53833

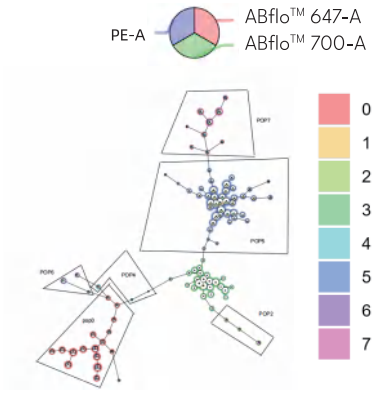
Automatic tSNE Analysis



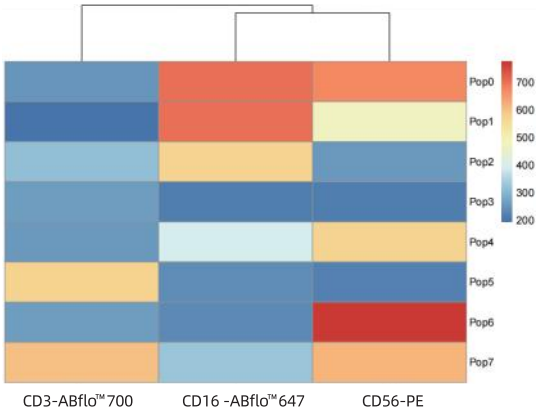
	Subset Name	Count
	FlowSOM.Live.Pop7.$CD3^+$ $CD56^+$ NKT cell	3550
	FlowSOM.Live.Pop5.$CD3^+$ $CD56^-$ T cell	24391
	FlowSOM.Live.Pop2.terminal NK	723
	FlowSOM.Live.Pop0.mature NK	8913
	FlowSOM.Live.Pop6.early NK	849
	Live	53833

Results: Within this plot, the Live cell population gated from Lymphocytes is depicted in red, early NK cells ($CD3^-$ $CD56^+$ $CD16^-$) in blue, mature NK cells ($CD3^-$ $CD56^+$ $CD16^+$) in orange, terminal NK cells ($CD3^-$ $CD56^-$ $CD16^+$) in green, $CD3^+$ $CD56^-$ T cells in dark green, and $CD3^+$ $CD56^+$ NKT cells in brown. Both the manual gating and FlowSOM automatic analysis consistently demonstrate accurate clustering and cell counts.

(5) FlowSOM Clustering Analysis



Results: The plot shows the clustering results obtained using FlowSOM's minimum spanning tree visualization. Cells sharing the same background color are assigned to the same cluster, while the size of the bubbles reflects the population size of each cell cluster. The pie charts provide valuable insights into the expression of the corresponding marker, with larger areas indicating higher expression and vice versa.



Results: The plot illustrates the FlowSOM Heatmap, with a color gradient ranging from red to blue indicating the expression of the corresponding markers. Within the heatmap, Pop0 represents the mature NK cell subset ($CD3^-$ $CD16^+$ $CD56^+$), Pop2 represents the terminal NK cell subset ($CD3^-$ $CD16^+$ $CD56^-$), Pop5 represents the $CD3^+$ $CD56^-$ T cell subset, Pop6 represents the early NK cell subset ($CD3^-$ $CD16^-$ $CD56^+$), and Pop7 represents the $CD3^+$ $CD56^+$ NKT cell subset. The heatmap provides valuable insights into the relative expression levels of these distinct cell subsets.

Flow cytometry antibodies related to NK cell phenotyping

Target	Fluorophore	Reactivity	Cat.No.
CD16	ABflo™ 488	Human	A23399
	ABflo™ 647	Human	A23400
CD56	PE	Human	A22826
	PE-Cy7	Human	A22829
	APC	Human	A22827
	APC-Cy7	Human	A22828
CD94	ABflo™ 488	Human	A23804
	ABflo™ 594	Human	A23806
	ABflo™ 647	Human	A23805
CD117	ABflo™488	Human	A22586

Target	Fluorophore	Reactivity	Cat.No.
CD117	ABflo™ 647	Human	A22587
	ABflo™ 488	Mouse	A23581
	ABflo™ 594	Mouse	A23583
	ABflo™ 647	Mouse	A23582
CD335	ABflo™ 488	Human	A24624
	ABflo™ 594	Human	A24626
	ABflo™ 647	Human	A24625
CD337	ABflo™ 488	Human	A23735
	ABflo™ 594	Human	A23737
	ABflo™ 647	Human	A23736

4. Antibodies Against Small Molecule

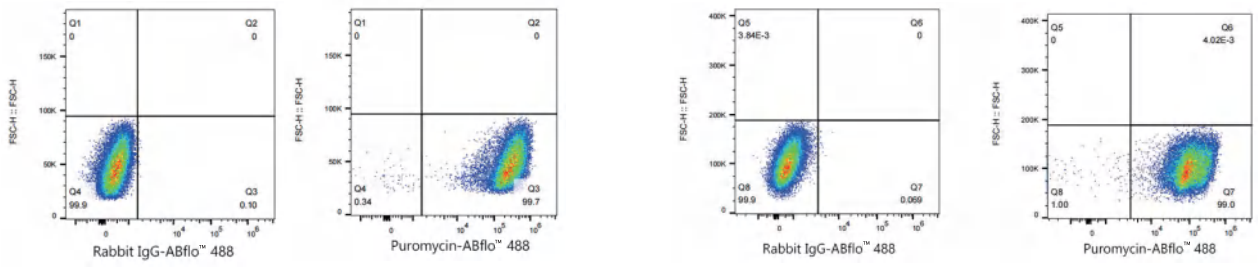
Puromycin plays a crucial role in detecting protein synthesis. It functions as a potent inhibitor of protein synthesis by integrating into the protein translation process. This integration causes premature termination of protein synthesis, resulting in the release of the puromycin-conjugated peptide from the ribosome. Flow cytometry, combined with antibodies specific to puromycin, enables the detection and analysis of protein synthesis. The measurement of protein synthesis levels provides insights into the overall cellular metabolic activity, making it a valuable indicator in cellular studies.

Target	Fluorophore	Reactivity	Cat.No.
Puromycin	ABflo™ 488	Species independent	A23130

Target	Fluorophore	Reactivity	Cat.No.
Puromycin	ABflo™ 647	Species independent	A23131

Case Study

ABflo™ 488 Rabbit anti-puromycin mAb (A23130)



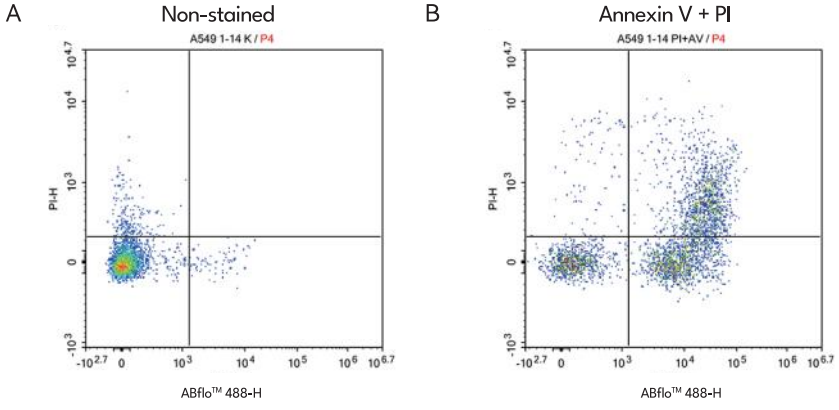
293T cells (treated with puromycin)

Raw264.7 cells (treated with puromycin)

5. Apoptosis Detection Kit

Case Study

ABflo™ 488 Annexin V/PI Apoptosis Detection Kit (RK05875)

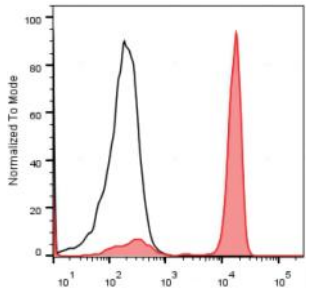


A549 cells

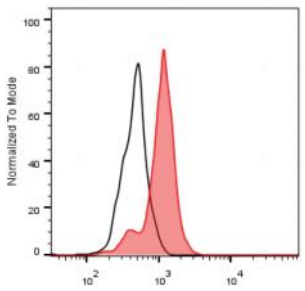
After treating A549 cells (human non-small cell lung cancer cells) with an apoptosis activator for 5 hours, the cells were either left unstained (A) or double-stained using ABflo™ 488 Annexin V and PI from this assay kit (B).

6. Customer Feedback

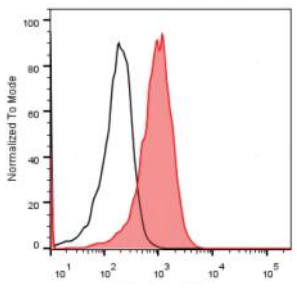
Institution: Medical Research Institute, Wuhan University
Research Area: Immunomodulation of Inflammation and Tumor Microenvironment
Samples: Human peripheral blood



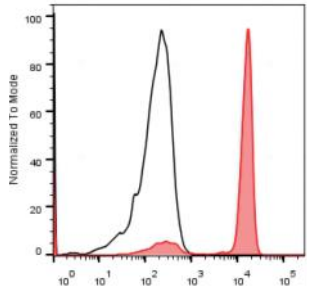
CD45-ABflo™ 488



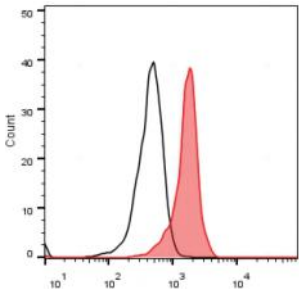
CD86-ABflo™ 488



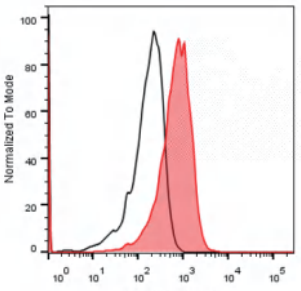
Siglec-8-ABflo™ 488



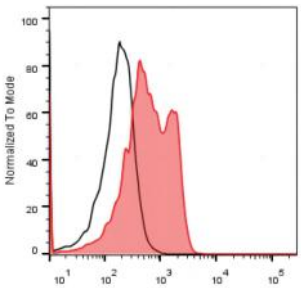
CD45-ABflo™ 647



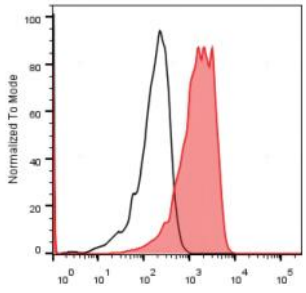
CD86-ABflo™ 647



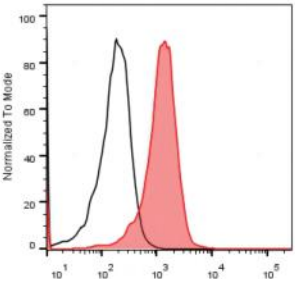
Siglec-8-ABflo™ 647



TIM-3-ABflo™ 488



TIM-3-ABflo™ 647



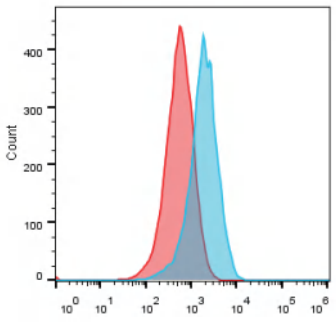
CD44-ABflo™ 488

Customer-Validated Antibodies

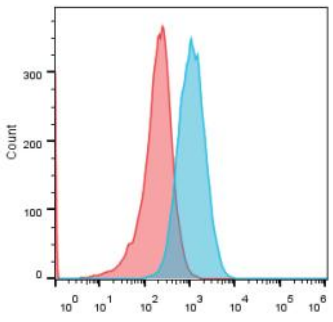
Target	Fluorophore	Reactivity	Cat.No.
CD45	ABflo™ 488	Human	A22494
	ABflo™ 647	Human	A22495
CD86	ABflo™ 488	Human	A21940
	ABflo™ 647	Human	A21941
Siglec-8	ABflo™ 488	Human	A22189

Target	Fluorophore	Reactivity	Cat.No.
Siglec-8	ABflo™ 647	Human	A22190
TIM-3	ABflo™ 488	Human	A22158
	ABflo™ 647	Human	A22489
CD44	ABflo™ 488	Human	A22519

Institution: Xiamen University
Research Area: Synthesis, screening, and structure-activity relationship study of natural medicine
Samples: Human cell lines



H460 cells



A549 cells

- Experimental Group: Cells plated overnight, pre-treated with LY294002 drug for 6 hours, followed by treatment with 10ng/mL IFN-γ recombinant cytokine for 72 hours, and cell collection.
- Control Group: DMSO

Detection Method: Flow cytometry
Detection Antibody: ABflo™ 488-conjugated PD-L1 antibody Antibody (Cat.No.: A22304)
Stimulating Protein: Human IFN-γ Active Protein (RP01038)

Customer-Validated Antibodies and Recombinant Proteins

Target	Fluorophore	Reactivity	Cat.No.
PD-L1	ABflo™ 488	Human	A22304

Target	Host	Reactivity	Cat.No.
IFN-γ	*E.coli*	Human	RP01038

Commonly Used Cytokines in Immune Cell Culture

Cat.No.	Cytokine	Species	Type
RP01216	M-CSF	Mouse	Macrophage M0
RP00183	M-CSF	Human	Macrophage M0
RP01161	IL-4	Mouse	Macrophage M2
RP01703	IL-4	Human	Macrophage M2
RP01465	IL-10	Mouse	Macrophage M2
RP00093	IL-10	Human	Macrophage M2

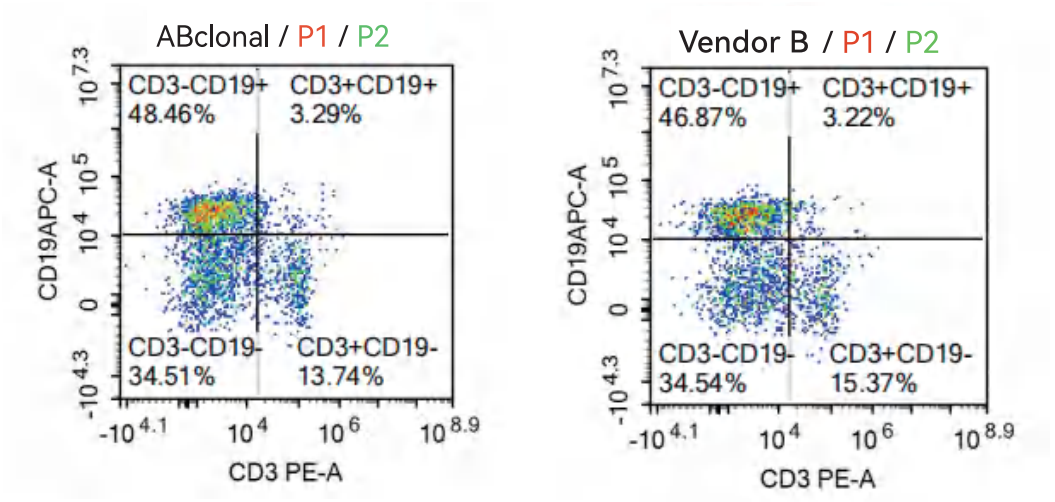
Cat.No.	Cytokine	Species	Type
RP01058	Flt3L	Mouse	DC
RP00175	Flt3L	Human	DC
RP01766	IL-7	Mouse	B cell
RP01040	IL-7	Human	B cell
RP01161	IL-4	Mouse	B cell
RP01703	IL-4	Human	B cell

ABflo™Novel Recombinant Rabbit Monoclonal Antibody for Flow Cytometry

Cat.No.	Cytokine	Species	Type
RP01596	IL-13	Mouse	Macrophage M2
RP01320	IL-13	Human	Macrophage M2
RP01070	IFN-γ	Mouse	Macrophage M1
RP01038	IFN-γ	Human	Macrophage M1
RP01161	IL-4	Mouse	DC
RP01703	IL-4	Human	DC
RP01206	GM-CSF	Mouse	DC
RP00094	GM-CSF	Human	DC
RP01071	TNF-α	Mouse	DC
RP00993	TNF-α	Human	DC
RP01458	TGF-β1	Human	TH17
RP01321	IL-6	Mouse	TH17
RP00201	IL-6	Human	TH17
RP02928LQ	IL-23	Mouse	TH17
RP01160	IL-23	Human	TH17
RP01384	IL-2	Mouse	Treg
RP01039	IL-2	Human	Treg
RP00671	TGF-β1	Mouse	Treg
RP01458	TGF-β1	Human	Treg
RP01321	IL-6	Mouse	Tfh
RP00201	IL-6	Human	Tfh

Cat.No.	Cytokine	Species	Type
RP01384	IL-2	Mouse	B cell
RP01039	IL-2	Human	B cell
RP02775LQ	IL-21	Mouse	B cell
RP01312	IL-21	Human	B cell
RP00018	BAFF	Human	B cell
RP01691	IL-12	Mouse	Th1
RP01232	IL-12	Human	Th1
RP01161	IL-4	Mouse	Th2
RP01703	IL-4	Human	Th2
RP00671	TGF-β1	Mouse	TH17
RP02775LQ	IL-21	Mouse	Tfh
RP01312	IL-21	Human	Tfh
RP01384	IL-2	Mouse	NK
RP01039	IL-2	Human	NK
RP01676	IL-15	Mouse	NK
RP01236	IL-15	Human	NK
RP01766	IL-7	Mouse	NK
RP01040	IL-7	Human	NK
RP02521	IL-18	Mouse	NK
RP00006	IL-18	Human	NK

Institution: Nanjing University
Research Area: Regulation of Inflammatory-Related Immune Microenvironment
Samples: Mouse liver



The results demonstrate that, when subjected to identical treatment conditions, ABclonal antibodies exhibit no significant difference in terms of fluorescence intensity and positivity rate when compared to competitor product B.

Customer-Validated Antibodies

Target	Fluorophore	Reactivity	Cat.No.
CD3e	PE	Mouse	A23321

Target	Fluorophore	Reactivity	Cat.No.
CD19	APC	Mouse	A23332

COMPANY PROFILE

Antibody | Protein | ELISA Kits | Enzyme | NGS | Service

ABclonal is an innovative growth company with the aim to provide reliable and costeffective products and services for both basic and translational research in the cutting edges of biomedical science. Innovation is our DNA. ABclonal has established R&D centers in the worldwide with different focuses to support technical innovation and product development for protein science and molecular biology. We always carefully listen to the opinions and feedback from talented scientists across the globe, quickly translate their needs into our product development pipeline, dedicating to develop the valuable research tools based on state-of-the-art technologies to meet the needs of innovation in a timely manner.

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