ClinMax™ Human TBNK 6-Color Cocktail Flow Panel

Catalog Number：ICA-A001

Assay Tests: 50 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product information

With premixed 6 kinds of fluorescent labeled antibodies (CD3, CD4, CD8, CD16/CD56, CD19, CD45), ClinMax™ Human TBNK 6-Color Cocktail Flow Panel is designed for use on flow cytometry to detect the

proportion of different immune cells in human peripheral blood lymphocyte samples in vitro.

Based on the biological function of cells and the antigens expressed on the cell surface, lymphocytes are divided into three main cell populations: T lymphocytes (CD3+), B lymphocytes (CD19+), NK cells (CD3-, CD16+ and/or CD56+). T lymphocytes are divided into helper T lymphocytes (CD3+CD4+) and killer T lymphocytes (CD3+CD8+) according to the expression of CD4 and CD8 on the surface. The percentage of T lymphocytes and B lymphocytes is used to monitor immunosuppression and autoimmune diseases. NK cells are a group of anti-tumor and virus-infected cells. NK cells-mediated cell killing not require antigen presentation and can directly act on target cells.

Introduction of the corresponding antigen characteristics:

CD3 molecule only exists on the surface of T cells, and the positive rate of CD3 in normal human peripheral blood lymphocytes and human thymocytes is 60-80 % and 60-70 %, respectively. In antigen recognition, CD3 antigen plays an important role in signal transduction.

CD4 molecule is a 55 KD type I single-chain transmembrane glycoprotein, mainly expressed in helper T (Th) cells, and is a co-receptor of Th cell TCR recognition antigen. Which binds to the non-peptide region of MHC class II molecules and participates in the signal transduction of Th cell TCR recognition antigen. 35-50 % of peripheral blood lymphocytes, helper and induced T cells, the antigen also express in low density on the surface of human monocytes.

CD8 molecule is a 68 KD type I transmembrane glycoprotein, linked by disulfide bonds, which includes a 32-34KD α chain and a 30-32 KD β chain. Most CD8 is a heterodimer, and the expression of peripheral blood is 15-40 % lymphocytes, that is, cytotoxic and inhibitory T cells-Ts/Tc. The CD8 molecule can bind to non-polymorphic portions of human histocompatibility complex class I (MHC-I) antigen and exert cytotoxic effects in a MHC-restricted manner.

CD16 molecule is a 50-65 KD low-affinity IgG Fc receptor (FcrRIII), expressed on a subset of most NK cells, granulocytes (except eosinophils), monocytes / macrophages, and T cells. CD16 antigen is mainly involved in the killing of target cells through the ADCC mechanism.

CD19 molecule is a 95 KD type I transmembrane glycoprotein and a B cell-restricted antigen, expressed throughout the whole process differentiation from B progenitor cells and maturation (disappearance on plasma cells), as well as malignant B cell tumor cells. CD19 antigen is positive in 8-20 % of normal peripheral blood lymphocytes, plays a regulatory role in B cell differentiation and development.

CD56 molecule is a 220-135 KD NK cell antigen, only expressed on large granular lymphocytes in peripheral

blood, which is actually a neuronal cell adhesion molecule isomer that mediates intercellular interactions. CD56 positive cells have natural killing activity.

CD45 molecule is a 180-220 KD transmembrane glycoprotein, abundantly expressed on the surface of all nucleated hematopoietic cells, such as bone marrow cells, thymocytes, lymphocytes, monocytes, granulocytes, also known as leukocyte common antigen (LCA). CD45 antigen plays an important role in T and B cell antigen receptor-mediated activation.

NOTE:

1. *This kit is research use only and is not used for diagnostic or therapeutic applications.*
2. *Please do not use the kit after the expiration date indicated on the kit labeling.*
3. *Protect fluorchrome labeled antibodies from light all times to prevent photobleaching.*
4. *Do not mix or substitute reagents with those from other lots or sources.*

Manufactured and distributed by

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Contents

Catalog	Amount	Status	Antibody Information			
			Antibody Marker	Clone	Immunoglobulin Subtypes	Fluorochrome
ICA-A001	50 tests	Powder	CD3	OKT3	IgG2a	FITC
			CD4	8C3	IgG1	PE-CY7
			CD8	OUCD8	IgG1	APC-CY7
			CD16	OUCD16	IgG1	PE
			CD19	FMC63	IgG2a	APC
			CD45	4A9.4	IgG2a	PerCp-Cy5.5
			CD56	OUCD56	IgG2b	PE

Storage

Keep the unopened kit at 2-8 °C. Avoid using the kit beyond its expiration date.

The opened kit and the reconstituted reagent should be stored at 2-8 °C and the shelf life is 30 days from the date of opening.

Required Materials not Supplied

Reagents	Single Staining & Compensation Controls	FITC-Labeled Monoclonal Anti-Human CD3 Antibody, Mouse IgG2a (Clone: OKT3)	FABm001-03
		PE-CY7-Labeled Monoclonal Anti-Human CD4 Antibody, Mouse IgG1 (8C3)	FABm002-04
		APC-CY7-Labeled Monoclonal Anti-Human CD8 Antibody, Mouse IgG	FABm003-05
		PE-Labeled Monoclonal Anti-Human CD16 Antibody, Mouse IgG	FABm005-01
		APC-Labeled Monoclonal Anti-Human CD19 Antibody, Mouse IgG2a (FMC63)	FABm004-02
		PerCP-Cy5.5 Labeled Monoclonal Anti-Human CD45 Antibody, Mouse IgG2a	FABm007-06
		PE-Labeled Monoclonal Anti-Human CD56 Antibody	FABm006-01
	Isotype Controls	FITC-Labeled Mouse IgG2a Antibody Isotype Control	DNP-FM487
		PE-CY7-Labeled Mouse IgG1 Antibody Isotype Control	FABm008-04
		APC-CY7-Labeled Mouse IgG1 Antibody Isotype Control	FABm008-05
		PE-Labeled Monoclonal Mouse IgG1 Antibody Isotype Control (Site-specific conjugation)	DNP-PM1
		APC-Labeled Mouse IgG2a Antibody Isotype Control	DNP-AFM487
		Percp-Cy5.5-Labeled Mouse IgG2a Antibody Isotype Control	FABm009-06
		PE-Labeled Mouse IgG2b Antibody Isotype Control	DNP-PM486

	/	Deionized, ultrapure or distilled water	/
	/	1×PBS	/
	/	BSA	/

Instrument	Multi-channel flow cytometer: the equipment configuration requirements are detailed in the following table
	Vortex oscillator
	Centrifuge with a 96-well plate rotator (for a 96-well plate samples preparation)
	Cell counter
Consumables	12×75 mm Flow tubes
	96-well Plate (flow instrument support 96-well plate testing and samples preparation for an auto-samples running)
	Pipette suitable for 2 μL, 10 μL, 100 μL, 1 mL and disposable pipette tip.

Flow Cytometer Configuration Requirements

≥2 Lasers	Laser/Ex Filter (nm)	Em Filter (nm)	Fluorochrome
Lasers and Filters	Blue (488)	~525	FITC
		~575	PE
		~695	PerCP-Cy5.5
		~785	PE-Cy7
	Red (~640)	~660	APC
		~785	APC-Cy7

≥3 Lasers	Laser/Ex Filter (nm)	Em Filter (nm)	Fluorochrome
Lasers and Filters	Blue (488)	~525	FITC
		~695	PerCP-Cy5.5
	Yellow-Green (~561)	~575	PE
		~785	PE-Cy7
	Red (~640)	~660	APC
		~785	APC-Cy7

Precaution

All chemicals should be considered as potentially hazardous. It is recommended that this kit is handled only by those persons who have been trained in laboratory techniques and it is used in accordance with the principles of good laboratory practice. Suitable protective clothing such as laboratory overalls, safety glasses and gloves is needed. Attention should be paid to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with plenty of water. All blood components and biological materials should be handled and disposed properly, in accordance with local and national guideline.

Working Buffers Preparation

For a repeatable detection assay, we recommend bringing the kit to room temperature and keep 15 minutes before use for a temperature balancing.

1. Fluorescent antibodies reconstruction: Add 275 μL ultrapure water to ClinMax™ Human TBNK 6-Color Cocktail Flow Panel (ICA-A001) and mix well, for completely dissolving, keep the bottle at room temperature for 15 minutes at least.
2. Stain buffer (1% BSA , 1×PBS): 0.5 g of BSA was dissolved in 50 mL of 1×PBS， which can be regulated according the samples used in your experiment.

Procedure (For Human PBMC)

1. Take the frozen PBMC from the liquid nitrogen tank and quickly resuscitate the cells in a 37°C water bath.
2. Resuspend the thawed PBMC cells with an appropriate volume of Stain buffer (for example, resuspending 1×10^7 cells with 6-10 mL Stain buffer), centrifuge for 5 min at 200 g, and discard the supernatant.
3. Resuspend PBMC precipitation with an appropriate volume of Stain buffer (for example, resuspending 1×10^7 cells with 5-6 mL Stain buffer), and count the cells with a cell counter.
4. Aspirate 2.5×10^5-1×10^6 cells/test, centrifuge for 5 min at 200 g, discard the supernatant, resuspend cells with 95 μL Stain buffer, pipette up and down, add in 5 μL of reconstituted fluorescent antibodies mixed solution and gently mix it well.

Note: *The recommended detection range of cells for this kit is from 2×10^4 to 1×10^6.*

5. Incubate for 15 min in the dark on ice, centrifuge for 5 min at 200 g. Discard the supernatant.
6. Resuspend cells with 200 μL Stain buffer and centrifuge for 5 min at 200 g. Discard the supernatant.
7. Repeat the washing step 6 once.
8. The cells were resuspended with 200 μL 1×PBS for each test. The sample is now ready to be analyzed on

the flow cytometer. If samples not be analyzed immediately, store them in the dark on ice. (For a live/dead staining, cells could be resuspended in pre-prepared live/dead dye DAPI).

Note:

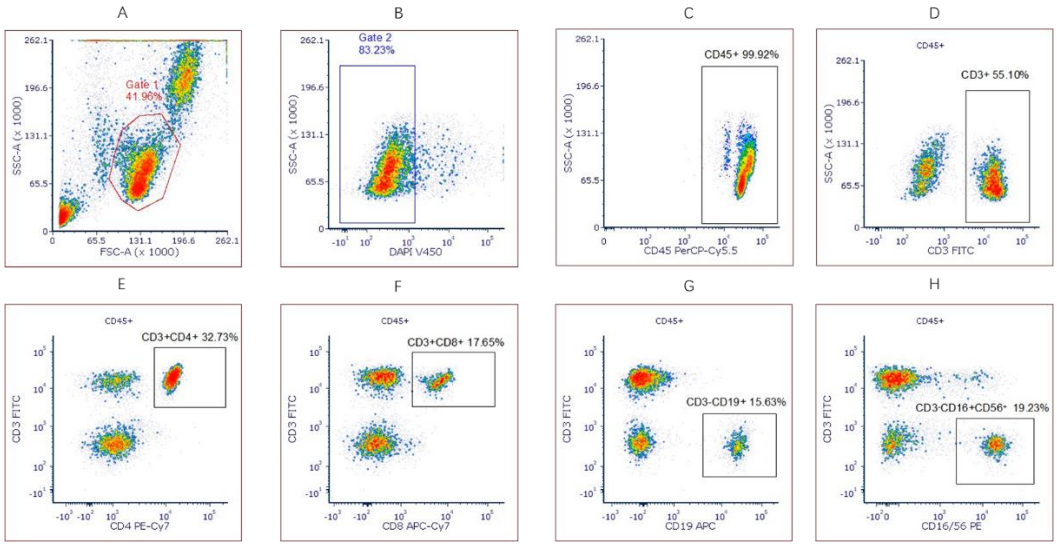
1） *After staining, the cells should be detected within 2 hours.*

2） *To prevent photobleaching, all the operations should try to avoid light.*

3） *For the overlap interruption of the spectrum Propidium Iodide (PI) and 7-AAD are not suggested cell staining for the usage of this kit*

Representative Data Analysis

As healthy people PBMC samples for an example, the data analysis is as follows:



TBNK cells gating logical and PBMC cell analysis

An FSC/SSC scatter plot was established for the total lymphocytes and gate as P1 (A). As lymphocytes cells with the P1 gate, set the P2 gate with DAPI/SSC scatter plot that assess the living cell population (B). As living cell population with P2 gate, set the CD45/SSC scatter plot, and the CD45+ gate assess the CD45+ lymphocyte cell population (C). Select the CD45+ gate cell population, set the CD3/SSC scatter plot to assess the CD3+ T cell population (D); the CD3/CD4 scatter diagram the Th gate assess the CD3+CD4+ helper T cell population (E); the CD3/CD8 scatter diagram the Tc gate assess the CD3+CD8+ toxic T cell population (F); the CD3/CD19 scatter diagram the B gate assess the CD3-CD19+ B cell population(G); and at last the CD3/CD16+56 scatter plot was set to assess the CD3-CD56+16+ NK cell population(H).

List of Compatible Flow Cytometers

Manufacturer	Instrument model	Laser filter	Em Filter and Fluorochrome
Beckman Coulter	Cytoflex S	488, 638, 561	B525-FITC, B690-Percp-CY5.5, R660-APC, R780-APC-CY7, Y585-PE, Y780-PE-CY7
BD	FACS Lyric	488, 640	FITC, Percp-CY5.5, PE, PE-CY7, APC, APC-Cy7
	FACSymphony A1	488, 637, 561	BB515-FITC, BB700- Percp-CY5.5, APC, APC-Cy7, PE, PE-CY7,
Thermo	Attune NxT	488, 637, 561	BL-1-FITC, BL-3-Percp-CY5.5, RL-1-APC, RL-3- APC-CY7, YL-1-PE, YL-4-PE-Cy7

Reference Range for Peripheral Blood Lymphocyte in Healthy Population

Lymphocyte subtype	Percentage contents（% of Lymphocyte）
CD3+	53.33%~81.22%
CD3+CD4+	24.01%~49.05%
CD3+ CD8+	15.71%~38.24%
CD3-CD19+	5.39%~18.23%
CD3-CD56+16+	6.37%~34.83%

Note： *Laboratories must establish their own normal reference intervals for ClinMax™ Human TBNK 6-Color Cocktail Flow Panel. parameters that can be affected by race, gender, age, individual variations of epitope expression and preparative technique. Reference intervals provided are for information only.*

Liu W, Xu J et. al. The reference ranges and characteristics of lymphocyte parameters and the correlation between lymphocyte parameters and routine health indicators in adults from China[J]. Immun Ageing, 2022, 19(1):42. DOI: 10.1186/s12979-022-00298-5