

EasyStain™ Human TBNK 6-color Cocktail Set 2

Catalog Number: E-AB-FC0025

Note: *Centrifuge before opening to ensure complete recovery of vial contents.*

Description

Reactivity	Human
Clone No.	UCHT1;B73.1;B-A19;HI30;HI19a;SK3;HIT8a
Conjugation	FITC;PE;PerCP;APC;PE/Cyanine7;APC/Cyanine7
Conjugation Information	FITC is designed to be excited by the Blue laser (488 nm) and detected using an optical filter centered near 530 nm (e.g., a 525/40 nm bandpass filter). PE is designed to be excited by the Blue (488 nm), Green (532 nm) and Yellow-Green (561 nm) lasers and detected using an optical filter centered near 575 nm (e.g., a 585/42 nm bandpass filter). PerCP is designed to be excited by the blue laser (488 nm) and detected using an optical filter centered near 675 nm (e.g., a 690/50 nm bandpass filter). APC is designed to be excited by the Red (627-640 nm) laser and detected using an optical filter centered near 660 nm (e.g., a 660/20 nm bandpass filter). PE/Cyanine7 is designed to be excited by the Blue (488 nm), Green (532 nm) and yellow-green (561 nm) lasers and detected using an optical filter centered near 775 nm (e.g., a 780/60 nm bandpass filter). APC/Cyanine7 is designed to be excited by the Red (627-640 nm) lasers and detected using an optical filter centered near 780 nm (e.g., a 780/60 nm bandpass filter).
Storage Buffer	Phosphate buffered solution, pH 7.2, containing 0.09% sodium azide.

Reagent Composition

The reagent contains the following conjugated antibodies:

Table 1 Reagent composition

Antibody	Fluorochrome	Clone	Isotype
CD45	PerCP	HI30	Mouse IgG1, κ
CD3	FITC	UCHT1	Mouse IgG1, κ
CD4	PE/Cyanine7	SK3	Mouse IgG1, κ
CD8	APC/Cyanine7	HIT8a	Mouse IgG1, κ
CD19	APC	HI19a	Mouse IgG1, κ
CD16	PE	B73.1	Mouse IgG1, κ
CD56	PE	B-A19	Mouse IgG1, κ

Applications

Recommended usage

FCM

1.1. For whole blood samples, add 5 µL EasyStain™ Human TBNK 6-color Cocktail Set 2 to 100 µL anticoagulant-treated blood sample in 2 mL centrifuge tubes.

NOTE: Peripheral blood samples, stored in EDTA or heparin sodium anticoagulation tubes, should be kept at 4°C for no more than 48 h. Before the experiment, a blood washing procedure is required. Take the required amount of peripheral blood and add an equal volume of PBS (1×). Mix well, centrifuge at 300g for 5 min, then remove the same volume of the supernatant, mix the sample again.

1.2. Mix and incubate the sample at 4°C in the dark for 25~30 min.

1.3. Add 1.9 mL of 1× ACK Lysis Buffer(E-CK-A105) and mix well. Incubate at 4°C for 10 min.

NOTE: 1× ACK Lysis Buffer is prepared freshly to remove red blood cells. For more details, please refer to the manufacturer's

For Research Use Only

instruction.

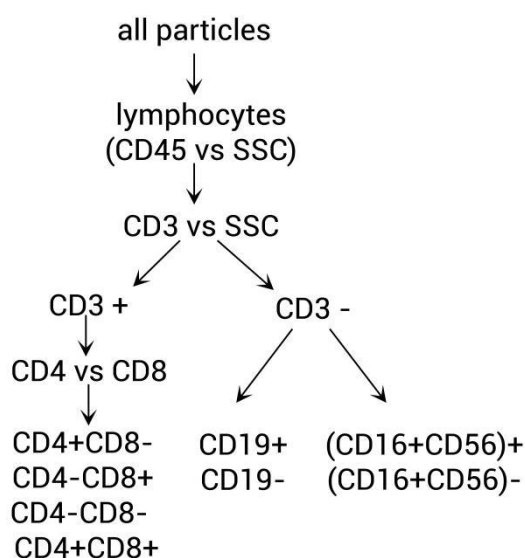
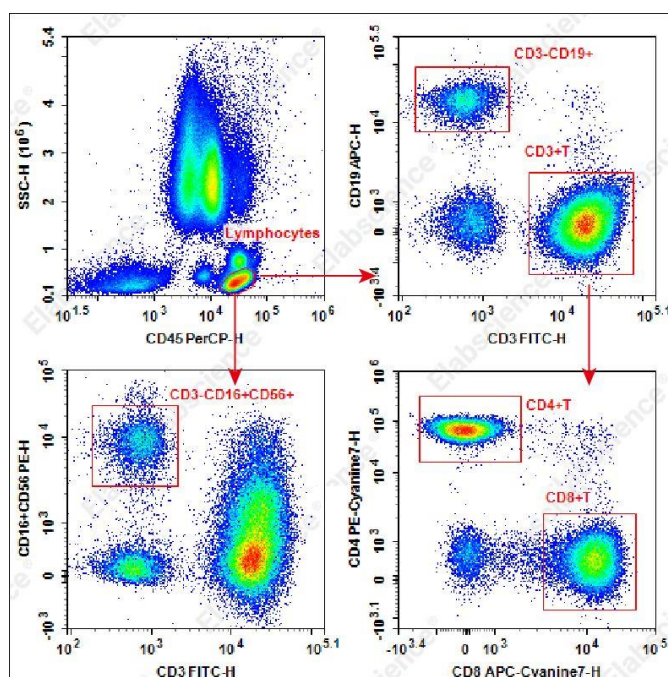
1.4. Centrifuge 300g for 5 min. Try to completely discard the supernatant. There will be visible white cell precipitates on the bottom and side walls of the tube.

NOTE: centrifuge immediately after lysis to prevent cell damage due to prolonged time

1.5. Resuspend the cells with 200 μ L cell staining buffer and load the sample on flow cytometer for detection.

NOTE: To avoid large particles in the sample, the cell suspension can be filtered through a 200-mesh sieve before detection. The sample is now ready to be analyzed on the flow cytometer. If samples will not be analyzed immediately after staining, store them in the dark at 4°C.

Data



Human peripheral blood lymphocytes are stained with EasyStain™

Human TBNK 6-color Cocktail Set 2.

Preparation & Storage

Storage

Keep as concentrated solution.

This product can be stored at 2-8°C for 24 months. Please protected from prolonged exposure to light and do not freeze.

Shipping

The product is shipped with ice pack, upon receipt, store it immediately at the recommended temperature.

Antigen Information

Background

This product is a FCM antibody cocktail made up of FITC Anti-Human CD3 Antibody[UCHT1] (Mouse IgG1, κ), PE anti-human CD16 Antibody[B73.1] (Mouse IgG1, κ), PE Anti-Human CD56/NCAM Antibody[B-A19] (Mouse IgG1, κ), PerCP Anti-Human CD45 Antibody[HI30] (Mouse IgG1, κ), APC Anti-Human CD19 Antibody[HI19a] (Mouse IgG1, κ), PE/Cyanine7 Anti-Human CD4 Antibody[SK3] (Mouse IgG1, κ), APC/Cyanine7 Anti-Human CD8a Antibody[HIT8a] (Mouse IgG1, κ).

CD3 ϵ is a 20 kD chain of the CD3/T cell receptor (TCR) complex, which is composed of two CD3 ϵ , one CD3 γ , one CD3 δ , one CD3 ζ (CD247), and a T cell receptor (α/β or γ/δ) heterodimer. It is found on all

For Research Use Only

mature T lymphocytes, NK T cells, and some thymocytes. CD3, also known as T3, is a member of the immunoglobulin superfamily that plays a role in antigen recognition, signal transduction, and T cell activation;

CD16 is known as low affinity IgG receptor III (FcγRIII). It is expressed as two distinct forms (CD16a and CD16b). CD16a (FcγRIIIA) is a 50-65 kD polypeptide-anchored transmembrane protein. It is expressed on the surface of NK cells, activated monocytes, macrophages, a subset of T cells and placental trophoblasts in humans. CD16b (FcγRIIIB) is a 48 kD glycosylphosphatidylinositol (GPI)-anchored protein. Its extracellular domain is over 95% homologous to that of CD16a, and it is expressed specifically on neutrophils. CD16 binds aggregated IgG or IgG-antigen complex which functions in NK cell activation, phagocytosis, and antibody-dependent cell-mediated cytotoxicity (ADCC);

CD56 is a single transmembrane glycoprotein also known as NCAM (neural cell adhesion molecule), Leu-19, or NKH1. It is a member of the Ig superfamily. The 140 kD isoform is expressed on NK and NKT cells. CD56 is also expressed in the brain (cerebellum and cortex) and at neuromuscular junctions. Certain large granular lymphocyte (LGL) leukemias, small-cell lung carcinomas, neuronal derived tumors, myelomas, and myeloid leukemias also express CD56. CD56 plays a role in homophilic and heterophilic adhesion via binding to itself or heparan sulfate;

CD45 is a 180 - 240 kD single chain type I membrane glycoprotein also known as leukocyte common antigen (LCA) and T200. It is a tyrosine phosphatase expressed on the plasma membrane of all hematopoietic cells, except erythrocytes or platelets. CD45 is a signaling molecule that regulates a variety of cellular processes including cell growth, differentiation, cell cycle, and oncogenic transformation. CD45 plays a critical role in T and B cell antigen receptor-mediated activation by dephosphorylating substrates including p56Lck, p59Fyn, and other Src family kinases. CD45 non-covalently associates with lymphocyte phosphatase-associated phosphoprotein (LPAP) on T and B lymphocytes. CD45 has been reported to bind galectin-1 and to be associated with several other cell surface antigens including CD1, CD2, CD3, and CD4;

CD19 is a 95 kD type I transmembrane glycoprotein also known as B4. It is a member of the immunoglobulin superfamily expressed on B-cells (from pro-B to blastoid B cells, absent on plasma cells) and follicular dendritic cells. CD19 is involved in B cell development, activation, and differentiation. CD19 forms a complex with CD21 (CR2) and CD81 (TAPA-1), and functions as a BCR co-receptor;

CD4, also known as T4, is a 55 kD single-chain type I transmembrane glycoprotein expressed on most thymocytes, a subset of T cells, and monocytes/macrophages. CD4, a member of the Ig superfamily, recognizes antigens associated with MHC class II molecules and participates in cell-cell interactions, thymic differentiation, and signal transduction. CD4 acts as a primary receptor for HIV, binding to HIV gp120. CD4 has also been shown to interact with IL-16;

CD8a is a 32-34 kD type I glycoprotein. It forms a homodimer (CD8a/a) or heterodimer (CD8a/b) with CD8b. CD8, also known as T8 and Leu2, is a member of the immunoglobulin superfamily found on the majority of thymocytes, a subset of peripheral blood T cells, and NK cells (which express almost exclusively CD8a homodimers). CD8 acts as a co-receptor with MHC class I-restricted T cell receptors in antigen recognition and T cell activation and has been shown to play a role in thymic differentiation. Two domains in CD8a are important for function: the extracellular IgSF domain binds the α3 domain of MHC class I and the cytoplasmic CXCP motif binds the tyrosine kinase p56 Lck;