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17-1015: Lineage Cocktail Assay kit (CD3 / CD14 / CD16 / CD19 / CD20 / CD56) FITC Conjugated

Clonality : Monoclonal
Clone Name : MEM 57, MEM-15, LNK16, LT19, LT20 and MEM-188
Application : FACS
Reactivity : Human
Format : Purified

Description

USE: The Lineage Cocktail (CD3 / CD14 / CD16 / CD19 / CD20 / CD56) FITC consists of the mixture of antibodies which stain human lymphocytes, monocytes, eosinophils and neutrophils. Peripheral blood dendritic cells and basophils can be identified by lack of staining with this reagent.
Principle: This test is based on specific binding of monoclonal antibodies to the antigenic determinants expressed on the surface of human lymphocytes, monocytes, eosinophils and neutrophils in peripheral blood. Dendritic cells and basophils can be identified in the peripheral blood by their lack of staining with this Lineage Cocktail using common flow cytometer device equipped with 488 nm laser. The monoclonal antibodies used in the Lineage Cocktail are labeled with Fluorescein isothiocyanate (FITC) which is excited by single laser beam from a flow cytometer during analysis. Staining of human blood cells is performed by incubation of blood samples with the Lineage Cocktail and, whenever applicable, the cells can be simultaneously stained by other monoclonal antibodies conjugated to appropriate fluorochromes. For example, dendritic cells can be distinguished from basophils by positive staining with PEconjugated anti-HLA-DR antibody. Staining whole peripheral blood with mixture of conjugated antibodies is followed by a lysis of red blood cells, wash and subsequent flow cytometry analysis.

Specificity: The monoclonal antibody MEM-57 reacts with CD3 complex, expressed on all peripheral blood T lymphocytes. The antibody MEM-15 reacts with CD14, expressed on monocytes, macrophages and weakly on neutrophils. The antibody LNK16 reacts with CD16, expressed on NK-cells, monocytes, macrophages and neutrophils. The antibody LT19 reacts with CD19, expressed on B lymphocytes. The antibody LT20 reacts with CD20, expressed on B lymphocytes. The antibody MEM-188 reacts with CD56, expressed on NK lymphocytes.

Product Info

Amount :	1 ml (50 Tests)
Content :	(PBS) containing 15mM sodium azide and 0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA) as a stabilizing agent.
Storage condition :	Store vial in the dark at 2-8°C. Do not freeze.

Application Note

Flowcytometry: 20 µl of the Lineage Cocktail / 100 µl of blood. However, this need to be optimized based on the research applications.

Staining protocol :

1. Collect peripheral blood in a sterile tube with an anticoagulant (e.g. Heparin, EDTA).
2. Add 20 µl of the Lineage Cocktail (CD3, CD14, CD16, CD19, CD20, CD56) FITC reagent to a test tube.

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3. Whenever applicable, add marker-specific antibody conjugated with appropriate fluorochrome to the test tube.
4. Add 100 µl of blood sample to the tube. Vortex the tube.
5. Incubate the tube for 20-30 minutes at room temperature in the dark.
6. Perform lysis of red cells using lysing solution. It is recommended to use a commercial lysing solution containing paraformaldehyde as a fixative. Follow the instructions of the lysing solution manufacturer.
7. Centrifuge the tube for 5 minutes at 300 g.
8. Remove supernatant and resuspend pellet with 3-4 ml of PBS.
9. Centrifuge the tube for 5 minutes at 300 g.
10. Remove supernatant and resuspend pellet with 0.3 – 0.5 ml of PBS.
11. Analyze the sample immediately using a flow cytometer or store sample at 2-8°C in the dark and analyze within 24 hours provided that cells were fixed.

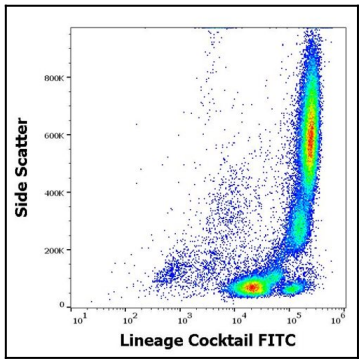

Figure-1: Flow cytometry surface staining pattern of human peripheral whole blood stained using Lineage Cocktail FITC (20 Î¼l reagent / 100 Î¼l of peripheral whole blood).

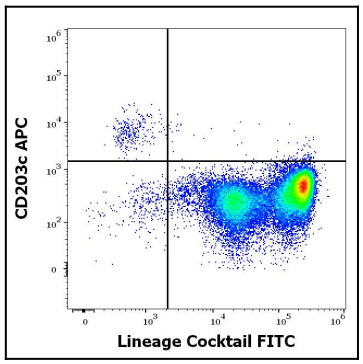

Figure-2: Flow cytometry multicolor surface staining of human peripheral whole blood stained using Lineage Cocktail FITC (20 Î¼l cocktail / 100 Î¼l of peripheral whole blood) and anti-human CD203c (NP4D6) APC antibody (10 Î¼l reagent / 100 Î¼l of peripheral whole blood).

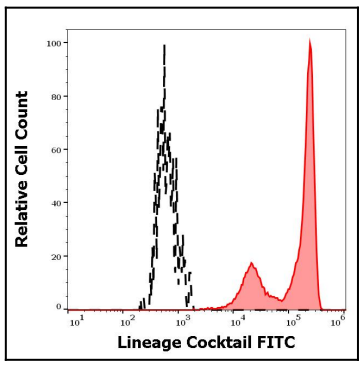

Figure-3: Separation of human target population cells (red-filled) from basophils (black-dashed) in flow cytometry analysis (surface staining) of human peripheral whole blood stained using Lineage Cocktail FITC (20 Î¼l cocktail / 100 Î¼l of peripheral whole blood).