EXBIO
Antibodies

Cat. No. 6K01-T050

Lineage Cocktail (CD3 / CD14 / CD16 / CD19 / CD20 / CD56) FITC

1. Specification

Content	50 tests, 1 ml					
Usage	20 µl per test					
Specificity	Hu CD3	Hu CD14	Hu CD16	Hu CD19	Hu CD20	Hu CD56
Clone	MEM-57	MEM-15	LNK16	LT19	LT20	MEM-188
Isotype	Ms IgG2a	Ms IgG1	Ms IgG1	Ms IgG1	Ms IgG2a	Ms IgG2a
Fluorochrome	FITC					
λ excitation	488 nm					
Emission maximum	525 nm					

2. Intended 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.

3. 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 PE-conjugated anti-HLA-DR antibody, cat. no. 1P-474-T100. Staining whole peripheral blood with mixture of conjugated antibodies is followed by a lysis of red blood cells, wash and subsequent flow cytometry analysis.

4. 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.

5. Reagent provided

The reagent contains premixed combination of mouse monoclonal antibodies against human CD3, CD14, CD16, CD19, CD20 and CD56 antigens (clone MEM-57, MEM-15, LNK16, LT19, LT20 and MEM-188, respectively) labeled with Fluorescein isothiocyanate (FITC). Labeled antibodies are diluted at optimum concentration in phosphate buffered saline (PBS) containing 15mM sodium azide and 0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA) as a stabilizing agent. The content of a vial (1 ml) is sufficient for 50 tests.

6. Storage

Store vial in the dark at 2-8°C. Do not freeze.

7. Precautions

- Intended for research use only.
- Do not use after expiration date stamped on vial label.
- Avoid prolonged exposure to light.
- The content of the vial must not freeze.
- Avoid contamination of the reagent.
- Any non-performance of staining protocol may produce false results.

- The reagent contains sodium azide (NaN_3) which is highly toxic in pure form. However, the concentration in the reagent (15mM) is not considered as hazardous. When disposing the reagent, flush the sink with large volume of water to avoid accumulation of explosive metal-azide in plumbing.
- Human blood samples are considered as potentially infectious and must be handled with care. Avoid all contact of the sample with the skin, eyes and mucosa.

8. Necessary material not supplied

Material necessary for collection of peripheral blood, test tubes for staining of blood samples (e.g. 12 × 75 mm), automatic pipettes with disposable tips, vortex mixer, centrifuge, commercial lysing solution, phosphate buffered saline (PBS), flow cytometer.

9. 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.
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.

10. Data analysis

Analyze the samples stained with the Lineage Cocktail (CD3, CD14, CD16, CD19, CD20, CD56) FITC reagent using a flow cytometer. If you measure simultaneously fluorescence signal from other fluorochrome, make correct compensation of fluorescent signals.

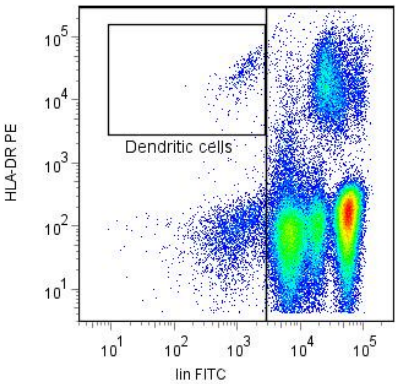

Fig. 1: Identification of dendritic cells in human peripheral blood by their lack of staining with Lineage Cocktail (CD3 / CD14 / CD16 / CD19 / CD20 / CD56) FITC and positive staining with anti-HLA-DR antibody conjugated with PE. 100 000 events were acquired for identification of HLA-DR$^+$ dendritic cells.

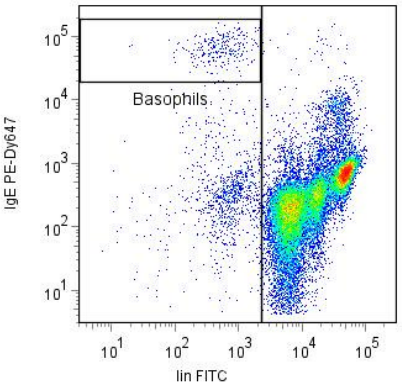

Fig. 2: Identification of basophils in human peripheral blood by their lack of staining with Lineage Cocktail (CD3 / CD14 / CD16 / CD19 / CD20 / CD56) FITC and positive staining with anti-IgE antibody conjugated with PE-DY647. 60 000 events were acquired for staining of IgE$^+$ basophils.

11. Limitations

- Flow cytometer may produce false results if the device has not been aligned and maintained appropriately.
- Data may be incorrectly interpreted if fluorescent signals were compensated wrongly or if gates were positioned inaccurately.
- Red blood cells from abnormal patients may be resistant to lysis using lysing solutions.
- In case of hyperleukocytose sample, it is recommended to dilute blood sample with PBS to obtain leukocyte density approximately 5 × 10^6 leukocytes/ml.
- Blood samples should be stained and analyzed within 24 hours from the blood collection.

12. References

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13. Manufacturer

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