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Monoclonal Antibody to CD4, FITC conjugated (CD4 FITC)

IVD CE

Cat. No.: ED7013

1. Specification

Specificity	Human CD4
Fluorochrome	FITC
Clone	MEM-241
Isotype	Mouse IgG1
Content	100 tests, 2 ml
Usage	20 µl per test
λ excitation	488 nm
Emission maximum	525 nm

2. Intended use

The reagent CD4 FITC permits identification and enumeration of cell populations expressing human CD4 antigen in whole blood using flow cytometry.

3. Principle

This test is based on specific binding of monoclonal antibody to the antigenic determinant expressed on the surface of leukocytes. The monoclonal antibody is labeled with fluorochrome which is excited via laser beam from a flow cytometer during analysis. Subsequent emission of light from fluorochromes of each cell is collected and analyzed by a flow cytometer. The fluorescence intensity differences enable separation of cell subsets based on expression of analyzed antigen.

Specific staining of blood cells is performed by incubation of blood samples with the reagent followed by a lysis of red blood cells. Afterwards, unaffected leukocytes are subjected to analysis by a flow cytometer.

4. Specificity

The antibody MEM-241 recognizes CD4 coreceptor, a 55 kDa transmebrane glycoprotein of immunoglobulin family expressed on subsets of T lymphocytes (such as "helper" T-cells, $CD4^+$ regulatory T cells or $CD4^+CD8^+$ double-positive T cells) and also on monocytes, tissue macrophages and granulocytes. The monoclonal antibody MEM-241 was assigned to CD4 during the Human Leukocyte Differentiation Antigen workshop *(HLDA8 (HCDM) WS Code: M241).*

5. Reagent provided

The reagent contains mouse monoclonal antibody against human CD4 antigen (clone MEM-241) which was purified by affinity chromatography and labeled with Fluorescein isothiocyanate (FITC). The labeled antibody is diluted in an optimal 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 (2 ml) is sufficient for 100 tests.

6. Storage

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

7. Precautions

- Intended for professional 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.
- 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), isotype control antibody (mouse IgG1 FITC), flow cytometer.

9. Staining protocol

1. Collect peripheral blood in a sterile tube with an anticoagulant (e.g. Heparin, EDTA).

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2. Add 20 µl of CD4 FITC reagent to a test tube, and the necessary amount of isotype control to a control tube.
3. Add 100 µl of blood sample to each tube. Vortex the tubes.
4. Incubate tubes for 20-30 minutes at room temperature in the dark.
5. Perform lysis of red cells using lysing solution. It is recommended to use a commercial lysing solution containing formaldehyde as a fixative. Follow the instructions of the lysing solution manufacturer.
6. Centrifuge tubes for 5 minutes at 300 g.
7. Remove supernatant and resuspend pellet with 3-4 ml of PBS.
8. Centrifuge tubes for 5 minutes at 300 g.
9. Remove supernatant and resuspend pellet with 0.3 – 0.5 ml of PBS.
10. Analyze samples immediately using flow cytometer or store samples at 2-8°C in the dark and analyze within 24 hours provided that cells were fixed.

10. Data analysis

Analyze sample stained with CD4 FITC using a flow cytometer. Visualize recorded data on the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population as shown in figure 1. Then make a histogram of lymphocytes with FITC intensity on the x-axis as shown in figure 2. Separate positive and negative populations using appropriate gates and calculate the percentage of CD4 positive lymphocytes. The region corresponding to the negative population should be set up using control cells which were stained by isotype control antibody.

11. Expected values

Results obtained in different laboratories may vary. Each laboratory should establish a normal range of cell subsets using its own test conditions. In our laboratory, the reagent CD4 FITC was tested on 40 blood samples of healthy people. Obtained results are given in the table below.

Parameter	Mean (%)	SD	CV (%)
CD4+ lymphocytes	43.5	8.3	19.1

12. 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.
- Blood samples from abnormal patients may exhibit abnormal values of positive cells.
- 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.

Example data

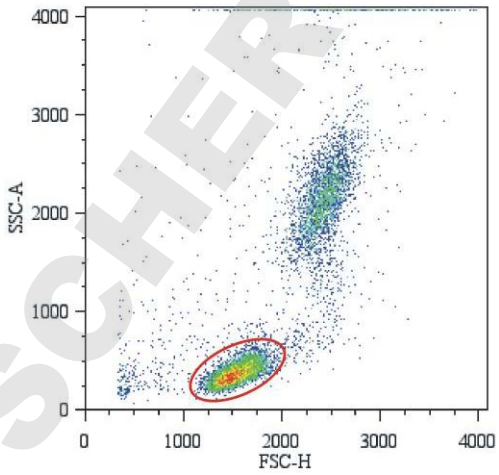

Fig. 1: Delimitation of lymphocyte population

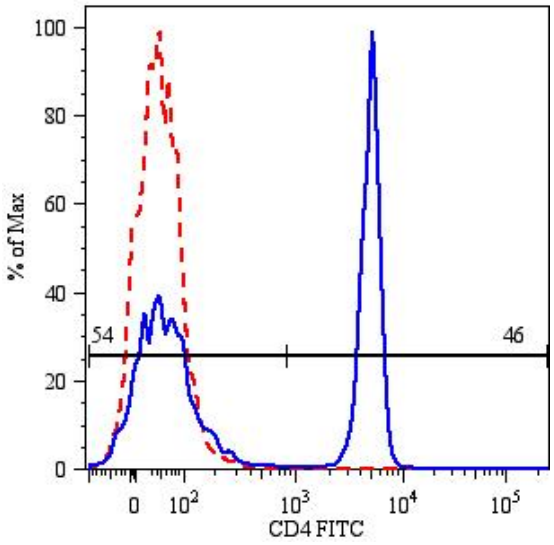

Fig. 2: Lymphocytes stained with CD4 FITC reagent

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