

Cat. No.	Clone	Form	Quantity	Presentation
1364	FMC7	FITC	100 tests	Liquid

PRESENTATION

This monoclonal antibody is supplied in a fluorescein isothiocyanate conjugated form, in 0.1M Tris-HCl, 0.5M NaCl, 1 mM glycine, 0.2% BSA, 0.1% NaN₃.

Each vial contains 1 ml of antibody solution pretitered for flow cytometry use (10 µl/test). This solution is ready to use.

The lyse and fix reagent is not provided with this format. It is available under cat. N°0486.

CHARACTERISTICS OF THE CLONE FMC7

- Immunogen : HRIK, a human B-lymphoblastoid cell line.
- Antigen : this antibody detects a glycoprotein of 105 kDa found on B-lymphocytes only.
- Antibody class : IgM (mouse).

STORAGE

Expiration date is indicated on the vial.
Store at 4°C before and after the opening of vials. DO NOT FREEZE.

DISTRIBUTION AND PROPERTIES

The gp 105 kDa antigen, recognized by the FMC7 antibody is expressed in a highly restricted way with the B lineage.
FMC7 positive normal B cells are more mature than FMC7 negative B cells.

The antigen is founded on B cell leukemias of the most differentiated stage such as prolymphocytic (PLL) and hairy cell (HCL) leukemias. It isn't expressed in most cases of chronic lymphocytic leukemia (CLL).

Reactivity occurs with peripheral blood B lymphocytes, and tonsil B lymphocytes, but not with granulocytes, monocytes, platelets, erythrocytes, T lymphocytes or null cells.

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Clinical evaluations

B cell prolymphocytic leukemia (B-PLL)	17/17
Hairy cell leukemia (HCL)	8/9
B cell chronic lymphocytic leukemia (B-CLL)	6/38

APPLICATIONS

This antibody is useful in the characterization of mature B cell leukemias by flow cytometry.

TECHNIQUES

DIRECT LABELLING ON WHOLE BLOOD WITH FITC CONJUGATE

In case of hyperleukocytosis, dilute the blood in an isotonic solution to obtain a leukocyte count of average value ($5000/\text{mm}^3$).

For a sample :

1. Pipet 100 μl of blood treated with EDTA into two tubes (1 test tube, 1 control tube).
2. In the test tube add 10 μl of the antibody. In the control tube add 10 μl of appropriate isotypic control or PBS. Vortex test and control tubes.
3. Incubate for 15 min. at room temperature.
4. Subsequently add 1 ml of lysing reagent (Cat N°0486) to the tubes and vortex. Wait 1 to 5 min. When the mixture becomes translucent, the lysing reaction is completed.

It is compulsory that the lysing reagent is used at 25°C.

5. Add 250 μl of the fixing reagent to each tube. Vortex the tubes.
6. Centrifuge the leukocyte preparation at 400 X g for 5 min.
7. Discard the supernatant cautiously (by aspiration).
8. Wash the pellet once with 3 ml PBS and centrifuge again, discard supernatant.
9. Add 100 to 500 μl of PBS or PBS formaldehyde 0.5% to the leukocyte pellet. The sample is ready for analysis.

LABELLING OF FICOLL HYPAQUE ISOLATED PBL

Recommended in case of leucopenia.

Cell enrichment

- 1) Dilute 5 ml of EDTA blood sample with 5 ml of isotonic or saline buffer.
- 2) Gently apply the diluted blood sample on a 5 ml Ficoll solution contained in a conical centrifuge tube.
- 3) Centrifuge for 15 min. at room temperature at 700 g.
- 4) Aspirate the mononucleated cells localized at the plasma-Ficoll interface using a "Pasteur" pipet.

- 5) Wash 3 times the collected mononucleated cells in 10 to 15 ml of Hank's medium or RPMI 1640 by centrifuging at 300 g during 10 min. at 4°C between each washing. Count the cells.
- 6) Eliminate the supernatant and resuspend the pellet in culture medium with 10% FCS. Adjust the cell concentration at about 5×10^6 cells /ml.

Direct cell labelling

For a sample :

- 1) Pipet 100 µl of cell suspension into two tubes (1 test tube, 1 control tube).
- 2) In the test tube add 10 µl of the antibody solution. In the control tube add 10 µl of appropriate isotypic control or PBS.
- 3) Incubate for 30 minutes at 4°C in the dark (temperature is very important).
- 4) Wash once with 3 ml PBS - Centrifuge and discard supernatant.
- 5) Resuspend the cells in 100 to 500 µl of PBS or saline with 0.5% of formaldehyde.
- 6) Analyse with the cytofluorograph or fluorescence microscope.

N.B. It is recommended to analyse the leukocyte preparation within 24 hours, because long term storage of the preparation might induce changes in the fluorescence properties of the cells.

REFERENCES

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