

Product datasheet for **AM39027RP-N**

Integrin alpha E (ITGAE) Mouse Monoclonal Antibody [Clone ID: B-ly7]

Product data:

Product Type:	Primary Antibodies
Clone Name:	B-ly7
Applications:	FC
Recommended Dilution:	Anti-CD103 (clone B-ly7) can be used in flow cytometry for analysis of blood and bone marrow samples, or in immunohistochemistry using frozen tissue sections. B-ly7 is a routinely applied marker for detection and follow-up of B cell malignancies. For the diagnosis of HCL, CD103 antibody (B-ly7) is frequently used together with CD19 antibody (clone HD37, cat-no. AM39014).
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Specificity:	Clone B-ly7 recognizes an integrin containing the α E subunit which dimerizes with the β 7 chain, present on hairy leukemia cells, to form the HML-1 (human mucosal lymphocyte) antigen. Clone B-ly7 is strongly reactive with hairy cell leukemia (HCL), activated monocytes, a subset of activated T and B cells (subtype of B cell chronic lymphocytic leukaemia), but not with other B cell leukemias or lymphomas. Testing by flow cytometry using a 'lyse-wash' method on a cell suspension containing HCL cells showed the following values expressed in terms of % of the total cell count: Product code: AM39027FC-N (anti-CD103 FITC) Mean % positive: 91,3 S.D.: 3,30 % CV: 3,61



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Formulation:	0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3, 0.2% BSA, 0.09% sodium azide Label: PE State: Liquid purified Ig fraction Label: <u>Cat. No. Label EX-max (nm) / EM-max (nm)</u> : AM39027FC-N FITC 488 / 519 AM39027RP-N PE 488, 532 / 578 AM39027PC5-N 488, 532 / 695 AM39027PU-N Pure . /
Concentration:	lot specific
Purification:	Affinity chromatography
Conjugation:	PE
Storage:	Store the antibody undiluted at 2-8°C. Fluorochrome labelled product is photosensitive and should be protected from light.
Stability:	Shelf life: one year from despatch.
Gene Name:	integrin subunit alpha E
Database Link:	<u>Entrez Gene 2209 Human</u> <u>Entrez Gene 3682 Human P38570</u>
Background:	The function of the CD103 integrin is related to T cell interaction with epithelium and to T cell adhesion. CD103 is expressed primarily on intra-epithelial lymphocytes and on 1-2% of peripheral blood lymphocytes. Cellular expression: Hairy Cell Leukemia (strong), subset activated T and B cells, activated monocytes.
Synonyms:	Integrin alpha-E, Integrin alpha-IEL, HML-1 antigen, ITGAE
Note:	<ol style="list-style-type: none">1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion.3. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants. <p>Protocol: <u>Flow cytometry method for use with labelled (FITC, R-PE, APC, PerCP or PerCP-Cy5.5) monoclonal antibodies</u></p> <ol style="list-style-type: none">1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.2. Add to each tube 10 µl of labelled monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study). Vortex the tube to ensure thorough mixing of antibody and cells.3. Incubate the tube for 15 minutes at room temperature in the dark.

4. Add 100 μ l of a lyse reagent.
5. Incubate for 10 minutes at room temperature in the dark.
6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
8. Remove the supernatant and resuspend the cells in 200 μ l of PBS.
9. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Flow cytometry method for use with dual and triple combinations

1. Add 100 μ l of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.

For combinations with anti-kappa and/or anti-lambda Ig see **Application note** below.

2. Add to each tube 20 μ l of labelled monoclonal antibody combination. (Appropriate mouse Ig isotype control samples should always be included in any labelling study).

3. Vortex the tube to ensure thorough mixing of antibody and cells.

4. Incubate the tube for 15 minutes at room temperature in the dark.

5. Add 100 μ l of a lyse reagent and mix immediately.

6. Incubate for 10 minutes at room temperature in the dark.

7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.

8. Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.

9. Remove the supernatant and resuspend the cells in 200 μ l of PBS.

10. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Application note for anti-kappa and/or anti-lambda Ig combinations

Add 2 ml of PBS containing 0.001% (v/v) Heparin (prewarmed to 37°C) to the cell suspension Vortex, centrifuge (2 min at 300x g) and discard the supernatant.

Repeat this step twice.

Resuspend the pelleted blood cells in 100 μ l PBS, pH 7.2, containing 0.001% (v/v) Heparin.

Flow cytometry method for use with purified monoclonal antibodies

1. Add 100 μ l of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.

2. Add to each tube 10 μ l of purified monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study).

Vortex the tube to ensure thorough mixing of antibody and cells.

3. Incubate the tube for 15 minutes at room temperature in the dark.

4. Wash the labelled cells by adding 2 ml of PBS containing 0.001% (v/v) Heparin, vortexing and centrifuging (2 min 1000 x g) and discard the supernatant.

5. Add 50 μ l of appropriate dilution of F(ab)₂ Rabbit Anti Mouse IgG fluorescent conjugate (e.g. FITC or R-PE) in PBS containing 0.001% (v/v) Heparin to the tube. It is recommended that the tube is protected from light.

- Mix by vortexing and incubate for 15 minutes at room temperature in the dark.
- Add 100 μ l of a lyse reagent and mix immediately.
- Incubate for 10 minutes at room temperature in the dark.
- Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
- Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.
- Remove the supernatant and resuspend the cells in 200 μ l of PBS.
- Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

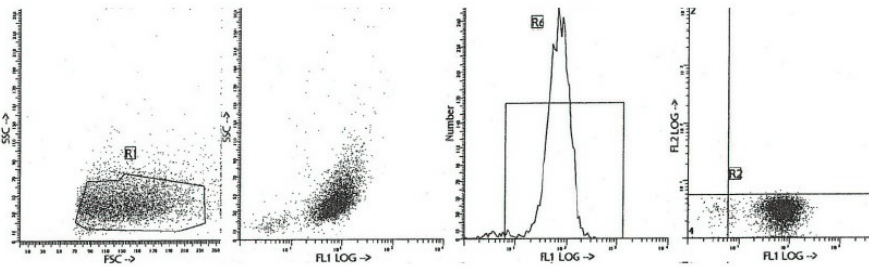
Protein Families:

Transmembrane

Protein Pathways:

Fc gamma R-mediated phagocytosis, Hematopoietic cell lineage, Systemic lupus erythematosus

Product images:



Representative Data Staining with clone B-ly7 (anti-CD103) monoclonal antibody is illustrated by flow cytometry analysis using a spleen cell suspension from a HCL patient. Direct staining was performed using 10 μ l of the FITC-conjugated antibody and 100 μ l spleen cell suspension.