OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for CL030P

Itga4 Rat Monoclonal Antibody [Clone ID: R1-2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	R1-2
Applications:	FC, FN, IHC, IP
Recommended Dilution:	Flow cytometry (see protocol). Immunoprecipitation. Immunohistochemistry on frozen sections. Functional assays.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Peyers Patch HEV binding lymphoma line (TK1)
Specificity:	This antibody reacts with alpha 4 integrin.
Formulation:	PBS buffer with 0.02% sodium azide as preservative State: Purified State: Liquid
Concentration:	lot specific
Purification:	Protein G affinity purified immunoglobulin fraction
Conjugation:	Unconjugated
Storage:	Store the antibody at 2-8°C for one month or at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	integrin alpha 4
Database Link:	Entrez Gene 16401 Mouse Q00651

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Background: Alpha 4 integrin, which helps to mediate cell-cell and cell-matrix interactions. It combines with beta 1 and beta 7integrin to form VLA-4 and LPAM-1 (Peyers patch homing receptor) respectively. VLA-4 is expressed on most peripheral lymphocytes, thymocytes and monocytes. LPAM-1 is found on peripheral lymphocytes, but few thymocytes. Fibronectin and VCAM-1 act as ligands for both VLA-4 and LPAM-1. LPAM-1 also binds the mucosal vascular addressin MAdCAM-1. (1)

Synonyms: Integrin alpha-4, Integrin alpha-IV, VLA-4, VLA4

Note: Protocol: FLOW CYTOMETRY ANALYSIS:

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 0.5-1.0 µg* of CL030P.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat anti-rat IgG (H+L)) at a 1/500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes.
(It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:

A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

N.B. Appropriate control samples should always be included in any labelling studies.