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Product datasheet for AM26037RP-L

Il2ra Rat Monoclonal Antibody [Clone ID: PC61.5.3]

Product data:

Product Type:	Primary Antibodies
Clone Name:	PC61.5.3
Applications:	FC
Recommended Dilution:	**Flow Cytometry:** 1.0 µg/10^6 cells (See Protoclols for more details).
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	B6.1 CTL cell line. Spleen cells from immunised OFA rats were fused with cells of the P3X63Ag8.653 mouse myeloma cell line.
Specificity:	This Monoclonal antibody reacts with the low affinity alpha chain of the interleukin-2 receptor present on activated T and B cells in mice. Clone PC61.5.3 is reported to inhibit IL-2 binding and IL-2 dependent proliferation.
Formulation:	PBS Label: PE State: Liquid purified IgG fraction Stabilizer: EIA grade BSA to bring total protein concentration to 4-5 mg/ml Preservative: 0.02% Sodium Azide
Purification:	Protein G Chromatography
Conjugation:	PE
Storage:	Store undiluted at 2-8°C. **DO NOT FREEZE!**
Stability:	Shelf life: one year from despatch.
Gene Name:	interleukin 2 receptor, alpha chain
Database Link:	Entrez Gene 16184 Mouse P01590

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Background: CD25 (IL2Ralpha, Tac) is a ligand-binding alpha subunit of interleukin 2 receptor (IL2R). Together with beta and gamma subunit CD25 constitues the high affinity IL2R, whereas CD25 alone serves as the low affinity IL2R. CD25 expression rapidly increases upon T cell activation. The 55 kDa CD25 molecule is enzymatically cleaved and shed from the cell surface as a soluble 45 kDa s-Tac, whose concentration in serum can be used as a marker of T cell activation. Expression of CD25 indicates the neoplastic phenotype of mast cells. CD25+ CD4+ FoxP3+ regulatory cells (Treg cells) play a crucial role in the control of organ-specific autoimmune diseases.

Synonyms: Interleukin-2 receptor alpha chain, IL-2 receptor alpha subunit, IL-2-RA, IL2-RA, p55, TAC antigen

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Note:

Protocol: **Flow Cytometry Analysis:**

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1x10^6$ cells, representing 1 test).
4. To each tube, add 0.5-1.0 µg of AM26037RP-S or AM26037RP-L per 10^6 cells.
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 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).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c
Cell Concentration: $1x10^6$ cells per tests.
Isotypic Control: Rat IgG1
Antibody Concentration Used: 1 µg /10^6 cells.
Isotypic Control: PE Rat IgG1.

Cell Source: Percentage of cells stained above control:

T Cell Blasts (Con A activated): 82.3%
Thymus (unactivated): 2.5%.

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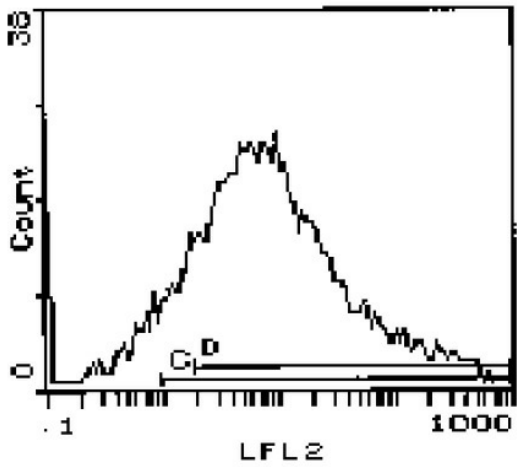
Product images:



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