

## Product datasheet for **AM26037PU-M**

### IL2ra Rat Monoclonal Antibody [Clone ID: PC61.5.3]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	PC61.5.3
Applications:	FC, FN, IHC, IP
Recommended Dilution:	<b>Flow Cytometry:</b> 1.0 µg/10 <sup>6</sup> cells (See Protocols for more details). <b>Immunoprecipitation.</b> <b>Functional Assays.</b> <b>Immunohistochemistry on Frozen Sections.</b>
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 State: Purified State: Liquid purified IgG fraction Preservative: 0.02% Sodium Azide
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	Unconjugated
Storage:	Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	interleukin 2 receptor, alpha chain



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**Database Link:** [Entrez Gene 16184 Mouse P01590](#)

**Background:** CD25 (IL2Ralpha, Tac) is a ligand-binding alpha subunit of interleukin 2 receptor (IL2R). Together with beta and gamma subunit CD25 constitutes 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

**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  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 1.0  $\mu$ g of AM26037PU-M.
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  $\mu$ l 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  $\mu$ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ l 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  $\mu$ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls).

**Results:****Tissue Distribution by Flow Cytometry Analysis:**

Mouse Strain: BALB/c

Cell Concentration:  $1 \times 10^6$  cells per tests.

Isotypic Control: Rat IgG1

Antibody Concentration Used: 1  $\mu$ g /  $10^6$  cells.

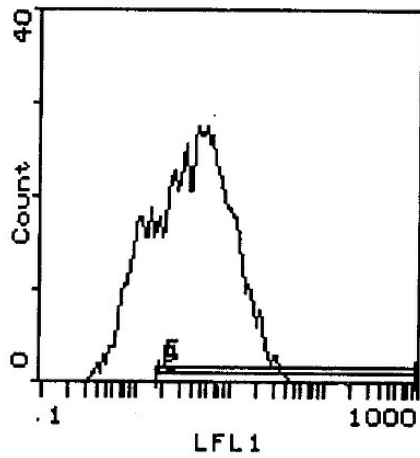
Isotypic Control: Rat IgG1.

**Cell Source: Percentage of cells stained above control:**

T Cell Blasts : 78.2%

Thymus (unactivated): 1.7%.

Product images:



Cell Source: T Cell Blasts (Con A activated).  
 Percentage of cells stained above control: 78.2%