

## Product datasheet for **CL042P**

### Kit Rat Monoclonal Antibody [Clone ID: ACK4]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	ACK4
Applications:	FC, IHC
Recommended Dilution:	<b>Flow Cytometry:</b> Use at 0.2 µg/ 10 <sup>6</sup> cells. Reported to be useful in immunohistochemistry on Frozen and Polyester Wax Sections.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	IL-3 dependent mast cells derived from WB- +/- mice. Donor: Wistar spleen. Fusion Partner: X63.653. Ag8.
Specificity:	This monoclonal antibody recognizes the receptor tyrosine kinase, c-kit. The ligand for this receptor is steel factor (stem cell factor), which exists in both soluble and membrane form. The interaction between steel factor and c-kit is essential for the development of hematopoietic, gonadal and pigment stem cells. c-kit positive cells are a subset of CD34+ hematopoietic precursor cells and it is expressed on 5-10% of total adult bone marrow cells.
Formulation:	PBS containing 0.02% Sodium Azide as preservative. State: Purified State: Liquid purified IgG fraction.
Concentration:	lot specific
Purification:	Protein G Chromatography.
Conjugation:	Unconjugated
Storage:	Store the antibody 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:	kit oncogene



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**Database Link:** [Entrez Gene 16590 Mouse P05532](#)

**Background:** c-Kit is a transmembrane tyrosine kinase encoded by the cKit proto oncogene. c-Kit acts to regulate a variety of biological responses including cell proliferation, apoptosis, chemotaxis and adhesion. Ligand binding to the extracellular domain leads to autophosphorylation on several tyrosine residues within the cytoplasmic domain, and activation. Mutations in c-Kit have been found to be important for tumor growth and progression in a variety of cancers including mast cell diseases, gastrointestinal stromal tumor, acute myeloid leukemia, Ewing sarcoma and lung cancer. Phosphorylation at tyrosine 721 of c-Kit allows binding and activation of PI3 kinase.

**Synonyms:** SCFR, KIT

## Note:

Protocol: **FLOW CYTOMETRY ANALYSIS:****Method:**

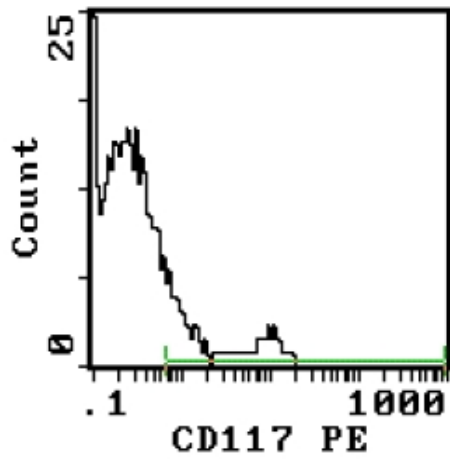
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 0.2-0.1  $\mu$ g of CL042P or CL042PX 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  $\mu$ l of PE Goat anti-Rat IgG (H+L) secondary antibody at appropriate 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:**Mouse Strain: BALB/cCell Concentration:  $1 \times 10^6$  cells per testAntibody Concentration Used: 0.2  $\mu$ g/ $10^6$  cellsIsotypic Control: Purified Rat IgG2a**Results - Strain Distribution:**Cell Concentration:  $1 \times 10^6$  cells per testAntibody Concentration Used: 0.2  $\mu$ g / $10^6$  cellsStrains Tested: AKR, BALB/c, C3H/He, C57BL/6Positive: AKR, BALB/c, C3H/He, C57BL/6Negative: none

Product images:



Cell Source: Bone Marrow. Percentage of cells stained above control: 7.3%