**For Research Use Only.
Not for use in diagnostic procedures.**

POLYCLONAL ANTIBODY

Anti-CD117 (c-Kit) (Human) pAb

Code No.	Quantity	Form
566	100 µL	Purified IgG

BACKGROUND: c-Kit, also known as stem cell factor receptor, steel factor receptor or CD117 is classified as a type III receptor tyrosine kinase (RTK) belonging to the platelet-derived growth factor receptor subfamily. Binding of stem cell factor (SCF), known as c-Kit ligand to c-Kit, initiates autophosphorylation of the receptor, subsequently leading to promotes a signal transduction cascade through Ras-Raf-MAP kinase cascade, phosphatidylinositol-3-kinase, src family kinases, and JAK/STAT pathways. The roles of c-Kit include maturation of hematopoietic and primordial germ cells precursors and melanocytes during embryonic development. In acute myeloid leukemia (AML), c-Kit has been proposed to play a functional role, and becomes target molecule for drug development.

SOURCE: This antibody was purified from rabbit serum using the synthesized peptides (C-terminal of c-Kit gene product; K963) coupled protein A agarose column. The rabbit was immunized with carrier protein conjugated synthesized peptides (K963).

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with c-Kit (145 kDa) on Western blotting and Immunohistochemistry.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; 1:200
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5)
Immunocytochemistry; 1:200
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Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Samples	HEL, pancreas	Not tested	Not tested
Reactivity on IHC/IC	+		

INTENDED USE:

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REFERENCES:

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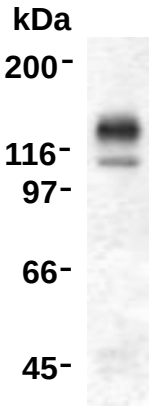
4) Lyford, G. L., *et al.*, *Gut* **51**, 496-501 (2002) [IHC]
5) Sakurai, S., *et al.*, *Am. J. Pathol.* **154**, 23-28 (1999) [IHC]
6) Tsuura, T., *et al.*, *Virchows Archiv.* **424**, 135-141 (1994)
7) Hidi, K., *et al.*, *Oncogene* **6**, 2291-2296 (1991)
8) Yarden,Y., *et al.*, *EMBO J.* **6**, 3341-3351 (1987)



Western blot analysis of c-kit expression in HEL cells using 566.

PROTOCOLS:

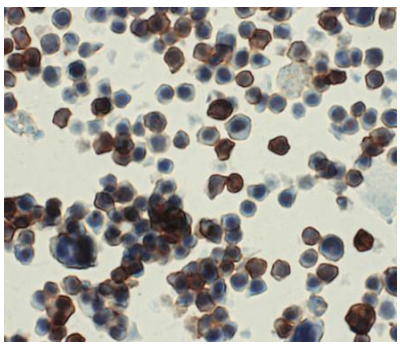
SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold

Lysis buffer to make 8 mg/mL solution.

3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; HEL)



Immunocytochemical detection of c-Kit on paraffin embedded section of HEL cells with 566.

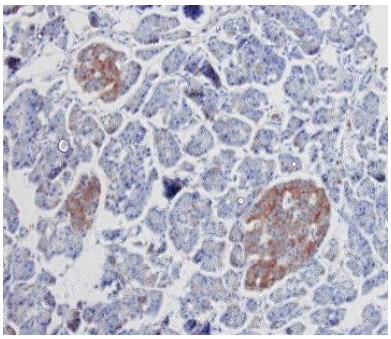
Immunocytochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment

Heat treatment by microwave oven:
Place the slides put on staining basket in 500 mL beaker with 500 mL citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.

5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with 1% BSA in PBS for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with ENVISION+Dual Link (Dako JAPAN; code no. 4063). Incubate for 1 hour at room temperature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with DAB substrate Kit (Dako JAPAN; code no. K3466). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.

(Positive control for Immunocytochemistry; HEL)



Immunohistochemical detection of c-Kit on paraffin embedded section of human pancreas with 566.

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment

Heat treatment by microwave oven:
Place the slides put on staining basket in 500 mL beaker with 500 mL citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.

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7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
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13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; Human pancreas)

RELATED PRODUCTS:

566-H	Anti-CD117 (c-Kit) (Human) pAb
PM035	Normal Rabbit IgG