Anti-c-Kit Antibody [PD00-24]

HA721154

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	IHC-P, WB, mIHC
Molecular Wt:	Predicted band size: 110 kDa
Clone number:	PD00-24

Description: CD117 is a 145-160 kDa cell membrane protein encoded by the c-kit proto-oncogene. CD117 is required for the development and growth of a large number of cells expressing this protein. The cells (particularly the mast cells) show a strong membrane as well as cytoplasmic staining. CD117 moreover is expressed in various epithelia (breast, sweat glands and salivary glands, renal tubular cells, thyroid follicular cells), usually showing a weaker, cytoplasmic reaction. CD117 is also demonstrated in testicular and ovarian interstitial cells, in neurons of the central nervous system (cerebellum, hippocampus, and dorsal horn of the spinal cord), and in immature myeloid cells. CD117 does not occur in smooth muscle cells. Family of transmembrane proteins essential for the regulation of cell growth and maintenance. When a growth factor is bound to its receptor, the latter is phosphorylated and begins a cascade of intracytoplasmic signals. Cells involved in the generation of electrical pacemaker activity for gastrointestinal motility. The cells are considered to be the origin of gastrointestinal stromal tumours. CD117 is of great importance for the classification of mesenchymal tumours of the gastrointestinal tract (including the mesentery). CD117 may also be used for classification of germinal cell tumours, as seminoma/dysgerminoma stains in the majority of cases, showing a strong membranous staining, while embryonal carcinoma stains in a small proportion of cases, with a weaker, membranous reaction. An panel for distinguishing seminoma from embryonal carcinoma should include CD30. Also in the identification of mast cell neoplasms CD117 has a potential. Appendix is recommended as positive and negative tissue controls for CD117.

Immunogen:	Synthetic peptide within Human c-Kit aa 950-976.
Positive control:	Human seminoma tissue, human gastrointestinal stromal tumor tissue, human breast tissue, Saos-2 cell lysate, human lung tissue lysate, human cervical cancer.
Subcellular location:	Cell membrane; Cytoplasm.
Database links:	SwissProt: P10721 Human
Recommended Dilutions:	
IHC-P	1:1,000
WB	1:1,000
mIHC	1:1,000
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

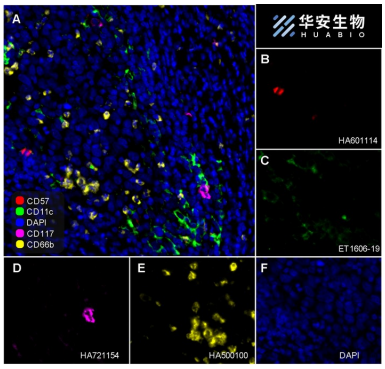

Fig1: Fluorescence multiplex immunohistochemical analysis of the human cervical cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD57 (HA601114, red), anti-CD11c (ET1606-19, green), anti-CD117 (HA21154, magenta) and anti-CD66b (HA500100, yellow) on human cervical cancer. Panel B: anti- CD57 stained on NKT cells. Panel C: anti-CD11c stained on dendritic cells. Panel D: anti-CD117 stained on mast cells. Panel E: anti-CD66b stained on neutrophils. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in four rounds of staining: in the order of HA601114 (1/500 dilution), ET1606-19 (1/1,000 dilution), HA721154 (1/1,000 dilution), and HA500100 (1/1,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

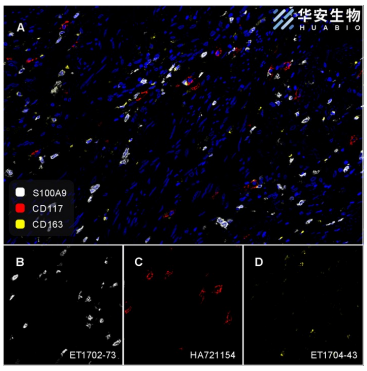

Fig2: Fluorescence multiplex immunohistochemical analysis of human cervical carcinoma (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-S100A9 (ET1702-73, White), anti-CD117 (HA721154, Red) and anti-CD163(ET1704-43, Yellow) on human cervical carcinoma. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1702-73 (1/1,000 dilution), HA721154 (1/1,000 dilution) and ET1704-43 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

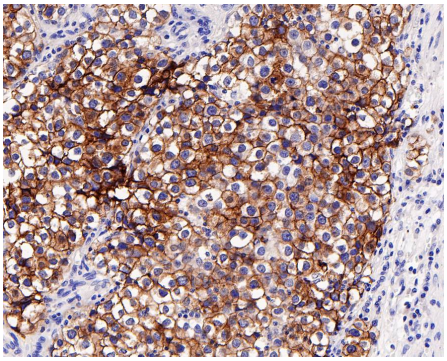

Fig3: Immunohistochemical analysis of paraffin-embedded human seminoma tissue with Rabbit anti-c-Kit antibody (HA721154) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721154) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

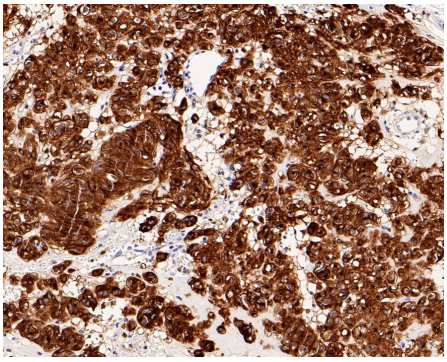

Fig4: Immunohistochemical analysis of paraffin-embedded human gastrointestinal stromal tumor tissue with Rabbit anti-c-Kit antibody (HA721154) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721154) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

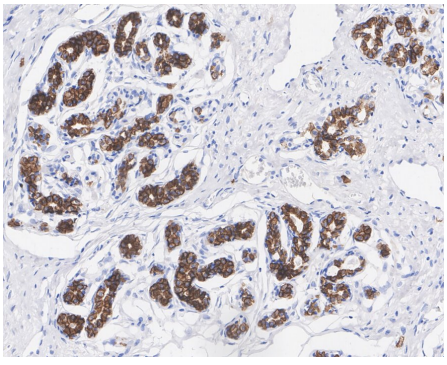

Fig5: Immunohistochemical analysis of paraffin-embedded human breast tissue with Rabbit anti-c-Kit antibody (HA721154) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721154) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

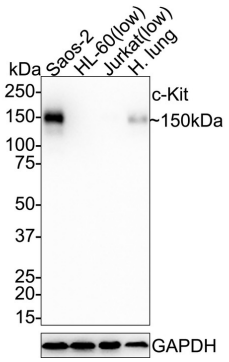

Fig6: Western blot analysis of c-Kit on different lysates with Rabbit anti-c-Kit antibody (HA721154) at 1/1,000 dilution.

Lane 1: Saos-2 cell lysate
Lane 2: HL-60 cell lysate (low expression)
Lane 3: Jurkat cell lysate (low expression)
Lane 4: Human lung tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 110 kDa
Observed band size: 150 kDa

Exposure time: 2 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721154) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

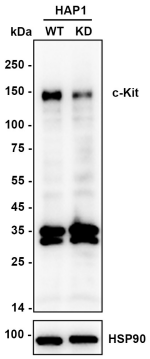

Fig7: Western blot analysis of c-Kit on different lysates with Rabbit anti-c-Kit antibody (HA721154) at 1/1,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-c-Kit KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 110 kDa
Observed band size: 150 kDa

Exposure time: 2 minutes 40 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721154) at 1/1,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Background References

1. Harris KS. et. al. CD117/c-kit defines a prostate CSC-like subpopulation driving progression and TKI resistance. Sci Rep. 2021 Jan
2. Russkamp NF. et. al. Anti-CD117 immunotherapy to eliminate hematopoietic and leukemia stem cells. Exp Hematol. 2021 Mar