

Anti-Human c-Kit Antibody [PSH26-40] - BSA and Azide free (Capture)

HA724619



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Cap)
Clone number:	PSH26-40

Description: Proto-oncogene c-KIT is the gene encoding the receptor tyrosine kinase protein known as tyrosine-protein kinase KIT, CD117 (cluster of differentiation 117) or mast/stem cell growth factor receptor (SCFR). Multiple transcript variants encoding different isoforms have been found for this gene. KIT was first described by the German biochemist Axel Ullrich in 1987 as the cellular homolog of the feline sarcoma viral oncogene v-kit. KIT is a cytokine receptor expressed on the surface of hematopoietic stem cells as well as other cell types. Altered forms of this receptor may be associated with some types of cancer. KIT is a receptor tyrosine kinase type III, which binds to stem cell factor, also known as "steel factor" or "c-kit ligand". When this receptor binds to stem cell factor (SCF) it forms a dimer that activates its intrinsic tyrosine kinase activity, that in turn phosphorylates and activates signal transduction molecules that propagate the signal in the cell. After activation, the receptor is ubiquitinated to mark it for transport to a lysosome and eventual destruction. Signaling through KIT plays a role in cell survival, proliferation, and differentiation. For instance, KIT signaling is required for melanocyte survival, and it is also involved in haematopoiesis and gametogenesis.

Immunogen: Recombinant protein within Human c-Kit aa 26-516 (HA211233).

Positive control: Recombinant Human c-Kit protein (HA211233).

Subcellular location: Cell membrane; Cytoplasm.

Database links: SwissProt: P10721 Human

Recommended Dilutions:

ELISA(Cap) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH26-41] to Human c-Kit (Detecor) (HA724620) or Rabbit monoclonal [PSH26-42] to Human c-Kit (Detecor) (HA724621) and Recombinant Human c-Kit protein (HA211233) as the standard. The reference range value is 11.7-3,000 pg/mL.

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

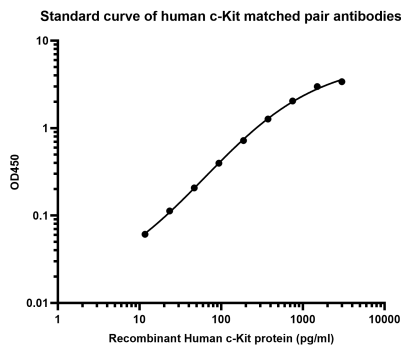
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Sandwich ELISA analysis of Human c-Kit matched pair antibodies

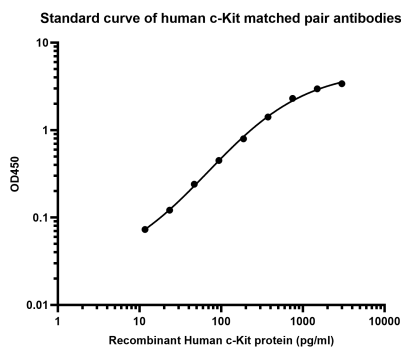
Capture: HA724619, Human c-Kit Rabbit mAb [PSH26-40]
Detector: HA724620, Human c-Kit Rabbit mAb [PSH26-41]



Elisa assay was performed by coating wells of a 96-well plate with 100 μ l per well of capture antibody (HA724619) diluted in carbonate/bicarbonate buffer, at a concentration of 2 μ g/mL overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150 μ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human c-Kit protein (HA211233) starting from 3,000 pg/mL to 0 pg/mL and detect antibody (HA724620, Biotin, 0.2 μ g/mL) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 100 μ l per well of SA-HRP for 0.5 hour at 30 $^{\circ}$ C with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

Fig2: Sandwich ELISA analysis of Human c-Kit matched pair antibodies

Capture: HA724619, Human c-Kit Rabbit mAb [PSH26-40]
Detector: HA724621, Human c-Kit Rabbit mAb [PSH26-42]



Elisa assay was performed by coating wells of a 96-well plate with 100 μ l per well of capture antibody (HA724619) diluted in carbonate/bicarbonate buffer, at a concentration of 2 μ g/mL overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150 μ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human c-Kit protein (HA211233) starting from 3,000 pg/mL to 0 pg/mL and detect antibody (HA724621, Biotin, 0.2 μ g/mL) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 100 μ l per well of SA-HRP for 0.5 hour at 30 $^{\circ}$ C with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

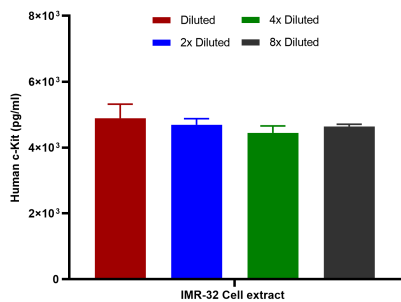


Fig3: Interpolated concentrations of native c-Kit in IMR-32 cell extract samples based on a 1,000 µg/mL extract load.

Capture: HA724619, Human c-Kit Rabbit mAb [PSH26-40]
Detector: HA724620, Human c-Kit Rabbit mAb [PSH26-41]

Interpolated concentration of native c-Kit was measured in duplicate at different sample concentrations. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean c-Kit concentration was determined to be 4,666 pg/mL in IMR-32 cell extract.

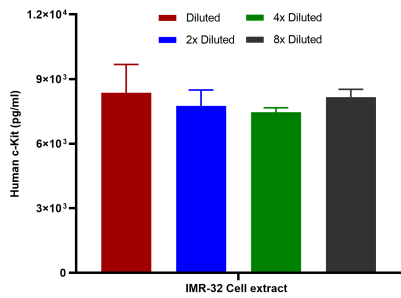


Fig4: Interpolated concentrations of native c-Kit in IMR-32 cell extract samples based on a 1,000 µg/mL extract load.

Capture: HA724619, Human c-Kit Rabbit mAb [PSH26-40]
Detector: HA724621, Human c-Kit Rabbit mAb [PSH26-42]

Interpolated concentration of native c-Kit was measured in duplicate at different sample concentrations. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean c-Kit concentration was determined to be 7,935 pg/mL in IMR-32 cell extract.

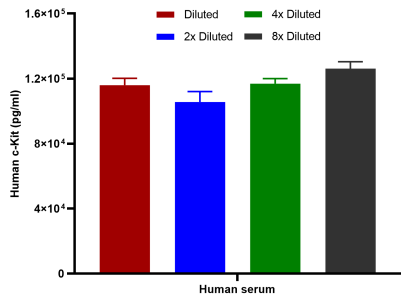


Fig5: Interpolated concentrations of native c-Kit in human serum.

Capture: HA724619, Human c-Kit Rabbit mAb [PSH26-40]
Detector: HA724620, Human c-Kit Rabbit mAb [PSH26-41]

Interpolated concentration of native c-Kit was measured in duplicate at different sample concentrations and interpolated from the c-Kit standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean c-Kit concentration was determined to be 116,136 pg/mL in human serum.

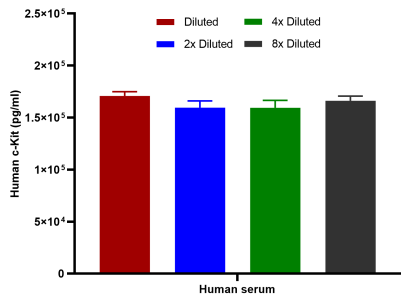


Fig6: Interpolated concentrations of native c-Kit in human serum.

Capture: HA724619, Human c-Kit Rabbit mAb [PSH26-40]
Detector: HA724621, Human c-Kit Rabbit mAb [PSH26-42]

Interpolated concentration of native c-Kit was measured in duplicate at different sample concentrations and interpolated from the c-Kit standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean c-Kit concentration was determined to be 163,987 pg/mL in human serum.

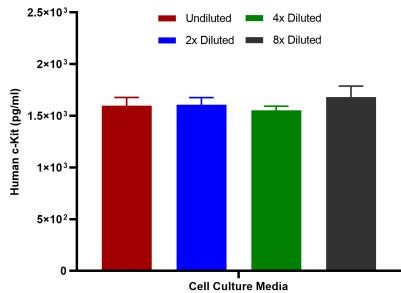


Fig7: Interpolated concentrations of spiked c-Kit in cell culture media samples.

Capture: HA724619, Human c-Kit Rabbit mAb [PSH26-40]
Detector: HA724620, Human c-Kit Rabbit mAb [PSH26-41]

The concentrations of c-Kit were measured in duplicates, interpolated from the c-Kit standard curves and corrected for sample dilution. diluted samples are as follows: 50% cell culture media with FBS. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

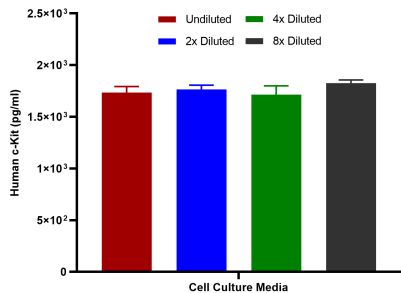


Fig8: Interpolated concentrations of spiked c-Kit in cell culture media samples.

Capture: HA724619, Human c-Kit Rabbit mAb [PSH26-40]
Detector: HA724621, Human c-Kit Rabbit mAb [PSH26-42]

The concentrations of c-Kit were measured in duplicates, interpolated from the c-Kit standard curves and corrected for sample dilution. diluted samples are as follows: 50% cell culture media with FBS. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

Note: All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

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

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation