Anti-Human Complement C5 Antibody [PSH06-45] - BSA and Azide free (Capture)

HA722682

Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human

Applications: ELISA(Cap)

Clone number: PSH06-45

Description: Activation of C5 by a C5 convertase initiates the spontaneous assembly of the late

complement components, C5-C9, into the membrane attack complex. C5b has a transient binding site for C6. The C5b-C6 complex is the foundation upon which the lytic complex is assembled. Derived from proteolytic degradation of complement C5, C5a anaphylatoxin is a mediator of local inflammatory process. Binding to the receptor C5AR1 induces a variety of responses including intracellular calcium release, contraction of smooth muscle, increased vascular permeability, and histamine release from mast cells and basophilic leukocytes. C5a is also a potent chemokine which stimulates the locomotion of polymorphonuclear leukocytes and directs their migration toward sites of inflammation. A rare defect of the complement classical pathway associated with susceptibility to severe recurrent infections, predominantly

by Neisseria gonorrhoeae or Neisseria meningitidis.

Immunogen: Recombinant protein within Human Complement C5 19-821.

Positive control: Recombinant standard Human Complement C5 protein (HA210636).

Subcellular location: Secreted.

Database links: SwissProt: P01031 Human

Recommended Dilutions:

ELISA(Cap) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit

monoclonal [PSH06-46] to Human Complement C5 antibody (Detector) (HA722683) and recombinant standard Human Complement C5 protein (HA210636). The reference range

value is 111-9,000pg/ml.

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at $+4^{\circ}$ C after thawing. Aliquot store at -20° C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Images

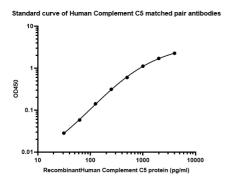


Fig1: Sandwich ELISA analysis of human Complement C5 matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 100 $\,\mu$ l per well of capture antibody (HA722682) diluted in carbonate/bicarbonate buffer, at a concentration of 5 $\,\mu$ g/ml overnight at 4°C. Wells of the plate were washed, blocked with 150 $\,\mu$ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted human Complement C5 protein starting from 4000 pg/ml to 0 pg/ml and detect antibody (HA722683, Biotin, 0.2 $\,\mu$ g/ml) for 1 hour at 30°C with shaking. Then the plate was washed and incubated with 100 $\,\mu$ l per well of SA-HRP for 0.5 hour at 30°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.

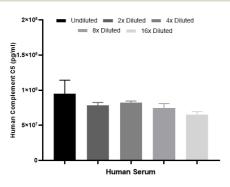


Fig2: Interpolated concentration of native Complement C5 was measured in duplicate at different sample concentrations. Undiluted samples were 1:10000 serum. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean Complement C5 concentration was determined to be 78.3 ug/mL in human serum.

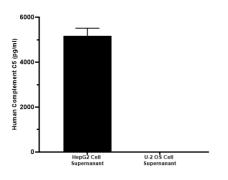


Fig3: Interpolated concentrations of native Complement C5 in human cell culture supernatant samples.

Complement C5 was measured in 100% cell supernatant. The concentrations of Complement C5 were interpolated from the Complement C5 standard curves. The mean Complement C5 concentration was determined to be 5.256 ng/mL in HepG2 cell supernanant. There was no detectable signal in U-2 OS cell supernatant.

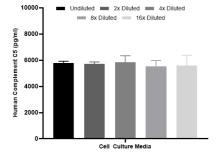


Fig4: Interpolated concentrations of spiked Complement C5 in human cell culture media samples.

The concentrations of Complement C5 were measured in duplicates, interpolated from the Complement C5 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

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

Background References

- 1. DeMartino J.A., Van Riper G., Siciliano S.J., Molineaux C.J., Konteatis Z.D., Rosen H., Springer M.S. The amino terminus of the human C5a receptor is required for high affinity C5a binding and for receptor activation by C5a but not C5a analogs. J. Biol. Chem. 269:14446-14450 (1994)
- 2. Hillebrandt S., Wasmuth H.E., Weiskirchen R., Hellerbrand C., Keppeler H., Werth A., Schirin-Sokhan R., Wilkens G., Geier A., Lammert F. Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. Nat. Genet. 37:835-843 (2005)