Anti-Human Von Willebrand Factor Antibody [PSH09-04] - BSA and Azide free (Detector)

HA723059



Species reactivity: Human

Applications: ELISA(Det)

Clone number: PSH09-04

Description:

Important in the maintenance of hemostasis, it promotes adhesion of platelets to the sites of vascular injury by forming a molecular bridge between sub-endothelial collagen matrix and platelet-surface receptor complex GPIb-IX-V. Also acts as a chaperone for coagulation factor VIII, delivering it to the site of injury, stabilizing its heterodimeric structure and protecting it from premature clearance from plasma. A common hemorrhagic disorder due to defects in von Willebrand factor protein and resulting in impaired platelet aggregation. Von Willebrand disease type 1 is characterized by partial quantitative deficiency of circulating von Willebrand factor, that is otherwise structurally and functionally normal. Clinical manifestations are mucocutaneous bleeding, such as epistaxis and menorrhagia, and prolonged bleeding after surgery or trauma. A hemorrhagic disorder due to defects in von Willebrand factor protein and resulting in altered platelet aggregation. Von Willebrand disease type 2 is characterized by qualitative deficiency and functional anomalies of von Willebrand factor. It is divided in different subtypes including 2A, 2B, 2M and 2N (Normandy variant). The mutant VWF protein in types 2A, 2B and 2M are defective in their plateletdependent function, whereas the mutant protein in type 2N is defective in its ability to bind factor VIII. Clinical manifestations are mucocutaneous bleeding, such as epistaxis and menorrhagia, and prolonged bleeding after surgery or trauma.

Immunogen: Recombinant protein within human von Willebrand factor protein aa 764-1263 (HA210992).

Positive control: Recombinant human von Willebrand factor protein (HA210992).

Subcellular location: Secreted.

Database links: SwissProt: P04275 Human

Recommended Dilutions:

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

monoclonal [PSH09-03] to Human Von Willebrand Factor antibody (Capture) (HA723058) and Recombinant Human Von Willebrand Factor protein (HA210992) as the standard. The

reference range value is 370-30,000 pg/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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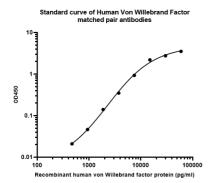


Fig1: Sandwich ELISA analysis of human Von Willebrand Factor matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 100 $\,\mu l$ per well of capture antibody (HA723058) diluted in carbonate/bicarbonate buffer, at a concentration of 5ug/ml overnight at $4^{\circ}{\rm C}$. 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 Von Willebrand Factor protein (HA210992) starting from 60000 pg/ml to 0 pg/ml and detect antibody (HA72359, Biotin, 0.2 $\,\mu g/ml)$ for 1 hour at $30^{\circ}{\rm C}$ with shaking. Then the plate was washed and incubated with 100 $\,\mu l$ per well of SA-HRP for 0.5 hour at $30^{\circ}{\rm C}$ 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.

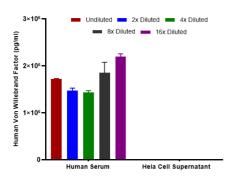


Fig2: Interpolated concentrations of native Von Willebrand Factor in human samples.

Interpolated concentration of native Von Willebrand Factor was measured in duplicate at different sample concentrations and interpolated from the Von Willebrand Factor standard curves. Undiluted samples were 2% human serum and 100% cell supernatant. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean Von Willebrand Factor concentration was determined to be 1.73 ug/mL in human serum. There was no detectable signal in Hela cell supernatant.

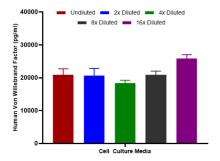


Fig3: Interpolated concentrations of spiked Von Willebrand Factor in cell culture media samples.

The concentrations of Von Willebrand Factor were measured in duplicates, interpolated from the Von Willebrand Factor 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).

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

Background References

- 1. Allen S., Abuzenadah A.M., Hinks J., Blagg J.L., Gursel T., Ingerslev J., Goodeve A.C., Peake I.R., Daly M.E. A novel von Willebrand disease-causing mutation (Arg273Trp) in the von Willebrand factor propertide that results in defective multimerization and secretion. Blood 96:560-568 (2000)
- 2. Bodo I., Katsumi A., Tuley E.A., Eikenboom J.C., Dong Z., Sadler J.E. Type 1 von Willebrand disease mutation Cys1149Arg causes intracellular retention and degradation of heterodimers: a possible general mechanism for dominant mutations of oligomeric proteins. Blood 98:2973-2979 (2001)