HUMAN VASPIN ELISA KIT

PURCHASE INFORMATION:

ELISA Name	Human Vaspin ELISA
Catalog No.	
Formulation	96 T
Standard range	0.08-50 ng/mL
Sensitivity	1.6 ng/mL
Sample Volume	50 µl
Sample Type	Serum, EDTA Plasma, cell culture
Specificity	Human Vaspin
Intra-assay Precision	4%
Inter-assay Precision	8%
Storage	2 °C-8 °C

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Human vaspin ELISA employs the quantitatively competitive enzyme immunoassay technique in which human vaspin present in samples competed with a fixed amount of biotinylated human vaspin for sites on purified rabbit IgG specific against human vaspin. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplates. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of human vaspin bound in the initial step. The sample values are then read off the standard curve.

Human vaspin ELSA has been shown to accurately quantitate the recombinant and natural human vaspin. Results obtained using natural human vaspin showed dose response curves that were parallel to the standard curves obtained using the kit standards.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Code	Quantity
R-Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with polyclonal IgG against rabbit IgG.	RMP01	1 plate
Vaspin Standard – 50 ng/vial of recombinant human vaspin in a buffered protein base with preservatives; lyophilized.	560-02-02	1 vial
Antibody– 350 µL / vial, 10-fold concentrated of polyclonal purified IgG against human vaspin with preservatives; lyophilized.	560-02-03	1 vial
Biotin Solution-350 µL / vial, 10-fold concentrated of human vaspin in a buffered protein base with preservatives; lyophilized	560-02-01	
Positive Control- one of recombinant human vaspin, lyophilized	560-02-04	1 vial
Streptavidin-HRP Conjugate -120 ul/vial, 100-fold concentrated solution of streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer- 30mL/vial of buffered protein based solution with preservatives	DB01	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution-13 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution（2N HCl）, 13 ml /vial of 2N HCl	S-STOP	1 vial
Plate Covers – Plate sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C. Do not use past kit expiration date.
Opened / Reconstituted Reagents: May be stored for up to 1 month at 2 - 8°C.
Standard should be stored for up to 1 month at -70° C.
Microplate Wells: Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

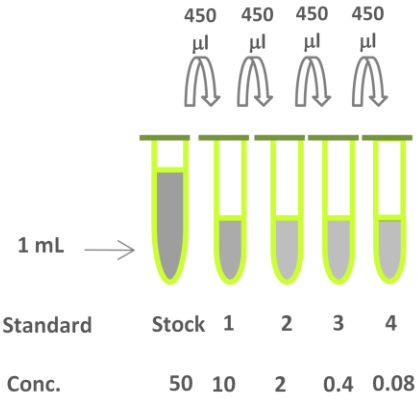
SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.
Human vaspin Standard - Refer to vial label for reconstitution volume. Reconstitute the **Human vaspin** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 50 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of the appropriate Dilution Buffer into the tube #1 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard.

Standard	Standard	Reagent Diluent	Concentration
Stock	Powder	1000 µl	50 ng/ml
# 1	50 µl of stock	450 µl	10 ng/ml
# 2	50 µl of 1	450 µl	2 ng/ml
# 3	50 µl of 2	450 µl	0.4 ng/ml
# 4	50 µl of 3	450 µl	0.08 ng/ml



Antibody- Reconstitute the **Antibody** with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 3.15 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 350 µl of 10-fold concentrated stock solution to prepare working solution.

Biotin Solution- Reconstitute the **Human vaspin biotinylated** with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 3.15 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 350 µl of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11. 88 mL of Dilution Buffer into the 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution.

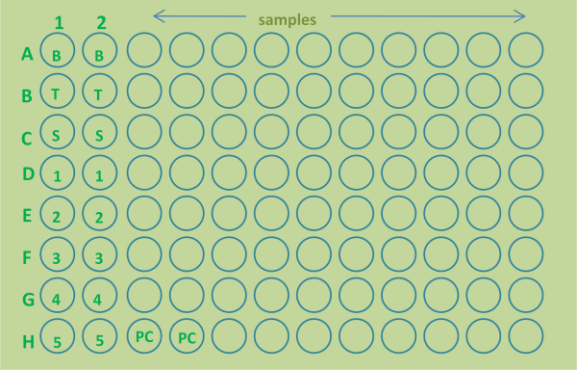
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. DO NOT ADD any Dilution Buffer, Antibody or Biotin Solution to Blank well (A1, A2).
4. Add 50 µL of Dilution Buffer to total Binding wells (B1, B2). Add 50 µL of Standard (from C1 to G2), sample, or control per well. Add 25 µL of Antibody Solution to each well. Cover with the Sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. *DO NOT aspirate and wash each well*. Add 25 µL of Biotin Solution to each well. Cover with the Sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
6. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (250 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature.
8. Repeat the aspiration/wash as in step 6.
9. Add 100 µL of Substrate Solution to each well. Incubate for 30-45 minutes at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 30 minutes, using a micro-plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human vaspin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATION

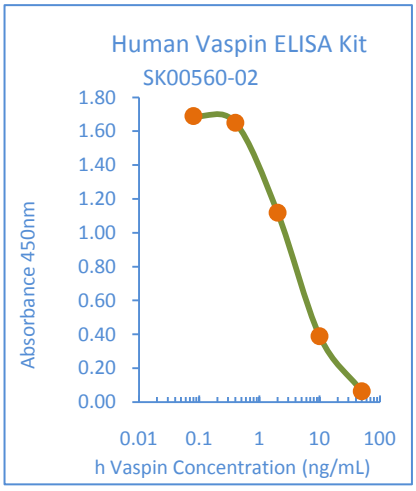
This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant human vaspin.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human vaspin was 1.6 ng/mL.

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
⇩
Add 50µl of standard, samples, positive control to each well. Add 25 µL of Antibody solution to each well. Incubate 2 hours on the plate shaker at RT.
⇩
Add 25 µl Biotinylated Protein to each well. Incubate 2 hours on the plate shaker at RT.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Streptatvin HRP conjugate to all wells. Incubate 1 hour on the plate shaker at RT.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Substrate to each well. Incubate 20-30min on the bench top. Protect from light.
⇩
Add 100 µl Stop Solution to each well. Read 450nm within 15 min



SPECIFICITY

This assay recognizes both natural and recombinant human vaspin. The data also indicated that human serum samples were competitively bound to antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. That means human serum samples cross-react with mouse vaspin ELISA kit.

Protein	Corss-reactivity
Human vaspin	100%
Mouse vaspin	100%
Human Visfatin	0
Human FABP-4	0
Human Leptin	0
Human gAdiponectin	0
Human Acrp30	0
Human FGF-21	0
Human CTRP3	0
Human Omentin 1	0
Human CTRP9	0
Human RBP-4	0
Human FTO	0
Human ADRP	0