V.2

Vaspin
ELISA Kit Instructions

For the quantitative determination of vaspin
in human serum

Catalog #80593
96 Assays

For research use only. Not for use in diagnostic procedures.

Crystal Chem, Inc.
955 Busse Road
Elk Grove Village, IL 60007, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
Order online: www.crystalchem.com

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A. Intended Use

The Vaspin ELISA kit is for the quantitative determination of vaspin in human serum. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Vaspin is a serine protease inhibitor of about 45.2 kDa. Its value as a marker is still unclear, but there does seem to be correlations of vaspin concentration to age, insulin sensitivity, glucose tolerance, certain cellular adhesion events, and visceral adipose tissue.

C. Principle of the Assay

The Vaspin ELISA kit is an ELISA sandwich assay for vaspin. It utilizes two specific and high affinity antibodies for this protein. Vaspin in the sample binds to the first antibody coated on the microtiter plate. In the following step, the biotinylated and Streptavidin-Peroxidase conjugated second specific anti-vaspin antibody binds in turn to the immobilized vaspin. In the closing substrate reaction, the vaspin levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the Vaspin ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD1-5	Standards (Lyophilized)	1 x 5 vials
CON1-2	Controls (Lyophilized)	1 x 2 vials
AB CONJ	Antibody Conjugate	1 x 12 mL
ENZ CONJ	Enzyme Conjugate	1 x 12 mL
DIL BUF	Dilution Buffer	1 x 120 mL
WASH	Wash Buffer (20X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
	Plate Seals	1 x 3

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled or deionized water
Polypropylene microtubes
Volumetric flasks
Vortex mixer
Microplate shaker (350 rpm)
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (50 µL), pipetting should be done as carefully as possible. A high quality 50 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Serum samples can be used. Samples should be chilled as soon as possible after sample withdrawal and stored in tightly closed tubes. If not used immediately, store samples at -20°C. Avoid repeated freeze-thaw cycles of samples.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture. Unused wells are stable for up to four weeks if stored at 2-8°C.
2. Standards 1-6
   Standards are provided in lyophilized form with concentrations ranging from 10 to 1000 pg/mL. Dilute each standard with 750 µL of Dilution Buffer. After reconstitution, it is recommended that standards be allowed to sit for 15 mins at room temperature and then mixed thoroughly but gently with a vortex mixer. Reconstituted standards are stable for 1 months at -20°C. Standards should be not be repeatedly thawed, so standards should be aliquoted in appropriate volumes prior to being frozen. Standards are provided in the following concentrations: 10, 75, 200, 500, and 1000 pg/mL.
3. Controls 1-2
   Controls are provided in lyophilized form with target value and ranges included on their labels. Dilute controls with 250 µL of Sample Buffer. After reconstitution, it is recommended that controls be allowed to sit for 15 mins at room temperature and then mixed thoroughly but gently with a Vortex mixer. Reconstituted controls are stable for 1 months at -20°C. Controls should be not be repeatedly thawed, so controls should be aliquoted in appropriate volumes prior to being frozen.
4. Antibody Conjugate
   Provides as ready to use.

5. Enzyme Conjugate
   Provided as ready to use.
6. Dilution Buffer
   Provided as ready to use.
7. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water. Wash buffer is stable for 4 weeks at 2-8°C after dilution, so dilute only as needed.
8. Substrate Solution
   Provided as ready to use. Protect from light.
9. Stop Solution
   Provided as ready to use.

I.2. Dilution of samples

Samples need to be diluted with Dilution Buffer for use with the assay. A sample dilution of 1:4 is generally suitable and should be performed as follows:

Dilute 1:4 by mixing 50 µL of sample with 150 µL of Dilution Buffer.

Since vaspin levels can vary, dilution ratio may need to be adjusted as appropriate. This kit shows good linearity to allow for sample dilutions from 1:2 to 1:32.

I.3. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample, standard, or control.
2. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
3. Aspirate well contents and wash five times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. Add 100 µL of the Antibody Conjugate in each well.
5. Cover the wells with sealing tape and incubate the plate for 1hr at room temperature (shake at 350 rpm).
6. Aspirate well contents and wash five times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of the Enzyme Conjugate in each well.
8. Cover the wells with sealing tape and incubate the plate for 1hr at room temperature (shake at 350 rpm).
9. Aspirate well contents and wash five times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
10. Add 100 µL of Substrate Solution in each well.
11. Incubate the plate for 30 mins in dark room at room temperature.
12. Stop the reaction by adding 100 µL of Stop Solution.
13. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.4. Determining the Vaspin concentration

1. Using computer software, construct the vaspin calibration curve by plotting the mean change in absorbance value for each calibrator on the Y axis versus the corresponding vaspin concentration on the X axis. A linear regression, four parametric logistic (4-PL) curve fit, or non-linear regression are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Vaspin concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor (ie. 4) to obtain the final vaspin concentration. The vaspin concentration is expressed in pg/mL.
   Note: *Samples with high vaspin concentrations (ie. fall above the range of the assay) should be further diluted with the Dilution Buffer and rerun.*

J. Performance characteristics

J.1. Assay range

The Vaspin ELISA Kit has an assay range from 10 – 1000 pg/mL.

J.2. Sensitivity

The analytical sensitivity of the assay is 4 pg/mL.

J.3. Precision

The assay has a within-run and total precision of CV < 10%.

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