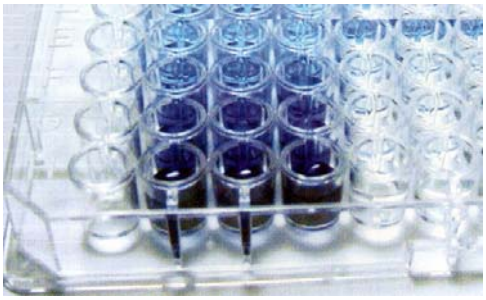
Human Vaspin ELISA Kit

Cat. No. V0712EK



Instruction Manual
Version 1.0.0

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

AdipoGen
Connecting Immunology to Metabolism

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Introduction

Vaspin, designated as **v**isceral **a**dipose tissue-derived **s**er**pin,** is a serpin whose expression is restricted to visceral adipose tissue. Its partial cDNA sequence was originally discovered as a upregulated gene expressed by obese Otsuka Long-Evans Tokushima Fatty (OLEFT) rats (1). Vaspin seems to retain the serpin signature conformation fold consisting of three b-sheets and nine a-helices as well as a reactive loop site that interacts with its cognate serine protease unidentified hitherto. Vaspin is remarkably upregulated at high-fat high-sucrose (HFHS) diet and at multiple metabolic dysfunctions like obesity or insulin resistance. Administration of TZD caused a significant induction of serum vaspin in mice. These facts imply that upregulation of vaspin may be a compensatory response to antagonize the action of other unknown proteases upregulated in obesity or insulin resistance. Indeed, administration of recombinant vaspin to obese mice fed with HFHS chow improved glucose tolerance and insulin sensitivity (1). Vaspin seems to directly act on white adipose tissue.

Assay Principles

This kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of vaspin in human serum, plasma or cell culture supernatants.

A monoclonal antibody specific for human vaspin has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any vaspin present is bound by immobilized antibody. Bound vaspin is captured by purified anti-human vaspin polyclonal antibody. HRP conjugated anti-rabbit IgG is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded vaspin quantity. The color development is stopped and the intensity of color is measured.

Kit Components

1) Antibody coated 96-well plate, 12 X 8-well strips
2) 5x Wash concentrate, 100 ml
3) 5x Diluent, 50 ml
4) Secondary antibody, 12 ml
5) 100x Detector, 150 µl
6) Standard, recombinant human vaspin expressed by HEK 293 cells, 1 vial, lyophilized
7) QC sample = recombinant human vaspin protein, 1 vial, lyophilized (For actual concentrations of QC sample, see the 'Certificate of analysis' enclosed.)
8) Substrate, 12 ml
9) Stop solution, 12 ml
10) Plate sealer, 3 sealers

Reagents Description

Antibody coated 96-well plate, 12 x 8-well strips, with absorbed monoclonal antibody against human vaspin
5x Wash concentrate, buffered detergent solution, supplied as a 5x concentrate
5x Diluent, for sample and reagent dilution
1x Secondary antibody, polyclonal antibody against human vaspin
100x detector, HRP conjugated rabbit IgG
Standard, 2.0 ng, 1 vial each, recombinant human vaspin, lyophilized
QC sample, 1 vial each, recombinant human vaspin protein
Substrate, chromogenic reagents
Stop solution, 1M H_3PO_4

Storage of Reagents

Reagent must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but not Supplied

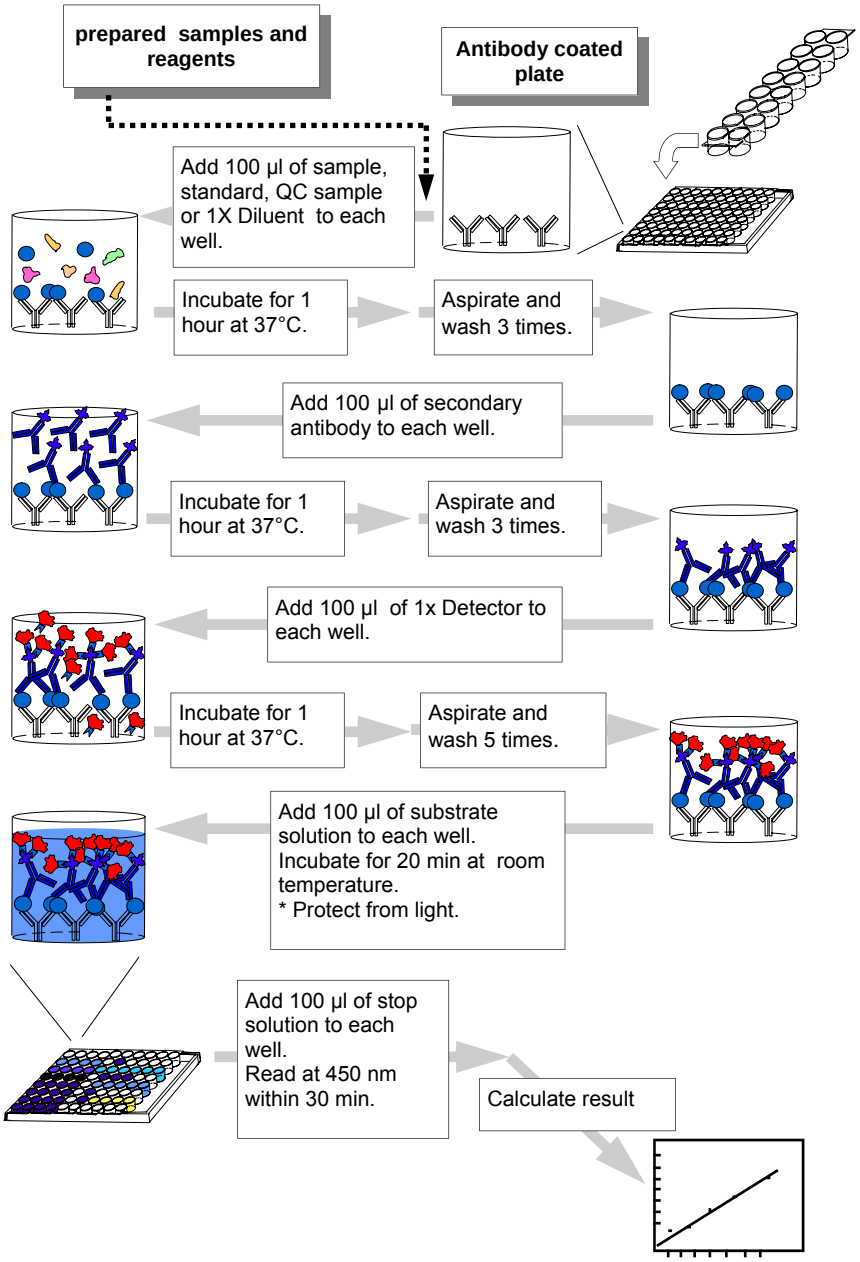
Precision single and multi-channel pipettes
Disposable pipette tips
Microtubes or equivalent for preparing dilutions
Disposable plastic containers for preparing working reagents
Reagent reservoirs
Microwell or microstrip plate reader 450 nm
Deionized water

Sample Collection and Storage

Serum Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ - 20^{o}C for later use. Avoid repeated freeze/thaw cycles.

Plasma Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ - 20^{o}C for later use. Avoid repeated freeze/thaw cycles.

Flow Chart of Assay Procedure



Assay Procedure

1. Preparation of Reagents

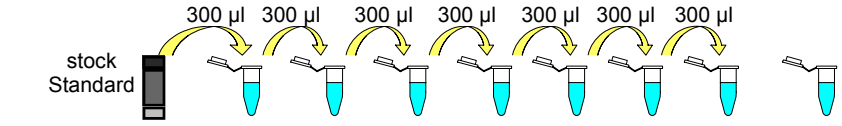
1) Allow all samples and kit components to equilibrate to room temperature (20-25°C).
2) Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use.
   It is recommended that Standards and samples be run in duplicate.
3) Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
4) Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
5) Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
6) Warm **Substrate Solution** to room temperature before use.
7) Prepare working aliquots of the Standard as follows :
   When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping.
   Add 1 ml of deionized water to the Standard vial to make a stock concentration of 2 ng/ml. Mix well.

A recommended dilution scheme is as follows :

A) Label 8 microcentrifuge tubes #0-7. Add 300 µl of the 1X Diluent to the microcentrifuge tubes # 0-7.

B) Add 300 µl of the stock Standard solution to tube # 7 and vortex. This is Standard tube # 7 with a concentration of 1 ng/ml.

C) Standards # 6 to # 1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube # 0.



Tube #	stock	7	6	5	4	3	2	1	0
Conc.(ng/ml)	2	1	0.5	0.25	0.125	0.063	0.031	0.016	0
Initial vol.(µl)	-	300	300	300	300	300	300	300	300

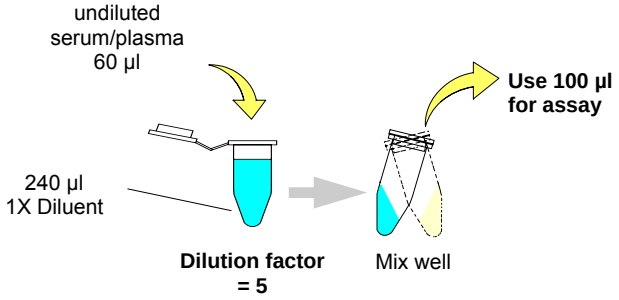
8) Reconstitute QC sample in 1 ml of deionized water. Mix well.

2. Sample Preparation

1) Dilute samples 1:5 with 1X Diluent (example, 60 µl sample plus 240 µl 1X Diluent; dilution factor=5) and mix well.

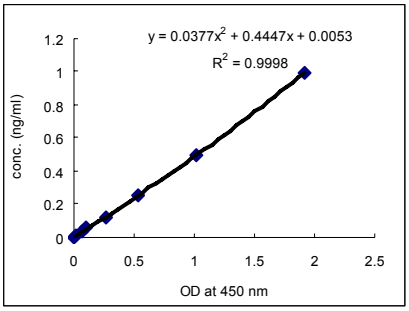
* If samples fall the outside range of assay, a lower or higher dilution may be required.

2) Use 100 µl of the final diluted sample for ELISA.



3. Experiment procedure

1) Remove the appropriate number of microwell strips from the sealed foil pouch.
2) Pipette 100 µl of standard # 0 to # 7, the reconstituted QC sample and diluted serum or plasma into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
3) Incubate at 37°C for 1 hour.
4) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
5) Add 100 µl Secondary Antibody to each well.
6) Incubate at 37°C for 1 hour.
7) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
8) Add 100 µl 1X Detector to each well.
9) Incubate at 37°C for 1 hour.
10) Remove the solution and wash 5 times with 300 µl of 1X Wash Solution to each well.
11) Add 100 µl of the Substrate Solution to each well.
12) Incubate at room temperature for 20 min.
    * Protect from light.
13) Using the multi-channel pipette, add 100 µl Stop Solution to each well.
14) Read at 450 nm.
15) Subtract the absorbance of the blank from the readings for each standard and sample.



16) Construct a standard curve by plotting the known concentrations (Y) of standard versus the absorbances (X) of standard. A measurable range is typically shown between 0.016 ng/ml and 1 ng/ml.
17) Calculate the vaspin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
18) The vaspin concentrations calculated must be multiplied by dilution factor [see **2. Sample Preparation**] to obtain the concentrations of the undiluted samples.

Performance Characteristics

1) Sensitivity : 12 pg/ml

2) Precision

a. Intra-Assay (precision within an assay)

5 samples were tested 6 times to assess intra-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	0.594	0.008	1.310
2	0.642	0.025	3.846
3	0.634	0.021	3.272
4	0.710	0.026	3.630
5	3.352	0.058	1.743

b. Inter-Assay (precision between assays)

5 samples were tested 6 times to assess inter-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	0.674	0.040	5.929
2	0.736	0.024	3.267
3	0.656	0.024	3.693
4	0.097	0.009	9.064
5	3.636	0.302	8.316

3) Specificity

a. No cross reaction with mouse and rat sera
b. Cross Reactivity

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Human vaspin	1	100
Human RBP4	10	N. R.
Human adiponectin	10	N. R.
Human visfatin	10	N. R.
Human leptin	10	N. R.
Human RELM β	10	N. R.
Human AGF	10	N. R.
Human FABP4	10	N. R.
Human TNF-α	10	N. R.
Human IL23p19	10	N. R.
Human GPX3	10	N. R.
Human IL-33	10	N. R.
Human ST2	10	N. R.
Human progranulin	10	N. R.
Human PAI1	10	N. R.
Mouse adiponectin	10	N. R.
Mouse resistin	10	N. R.

N. R.: No Cross-Reactivity

4) Recovery

The recovery of vaspin spiked to two different levels in four different human serum samples throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
1	97.70	94-101
2	100.07	97-103
3	100.82	98-104
4	99.06	90-107

5) Linearity - Effect of Serum Dilution

To assess the linearity of the assay, three serum samples were first diluted as indicated below prior to sample preparation as described in the protocol.

Sample No.	Serum Dilution	Expected (ug/ml)	Observed (ug/ml)	% Of Expected
1	1	0.547	0.547	100
	1/2	0.274	0.283	103
	1/4	0.137	0.149	109
2	1	0.429	0.429	100
	1/2	0.215	0.232	108
	1/4	0.107	0.117	109
3	1	0.601	0.601	100
	1/2	0.301	0.307	102
	1/4	0.150	0.152	101

% of expected = observed / expected x 100%

References

1. Hida, K., Wada, J., Eguchi, J. et al. 2005 Visceral adipose tissue-derived serine protease inhibitor: A unique insulin-sensitizing adipocytokine in obesity. *Proc. Natl. Acad. Sci.* 102: 10610-10615.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Troubleshooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

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