SERPINA12 (Human/Mouse/Rat) ELISA Kit

Catalog Number KA1688

96 assays

Version: 02

Intended for research use only

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Introduction

Background

Visceral adipose tissue-derived serpin (Vaspin), a member of serine protease inhibitor family, is a visceral adipose tissue-derived adipokine with potential antiprotease properties.

Vaspin cDNA was isolated by from visceral white adipose tissues (WATs) of Otsuka Long-Evans Tokushima fatty (OLETF) rat, an animal model of abdominal obesity with type 2 diabetes. Human, mouse and rat Vaspin is made up of 395, 394, and 392 amino acids respectively. Vaspin belongs to serine protease inhibitor family and it also shows about 40% homology with 1-antitrypsin.

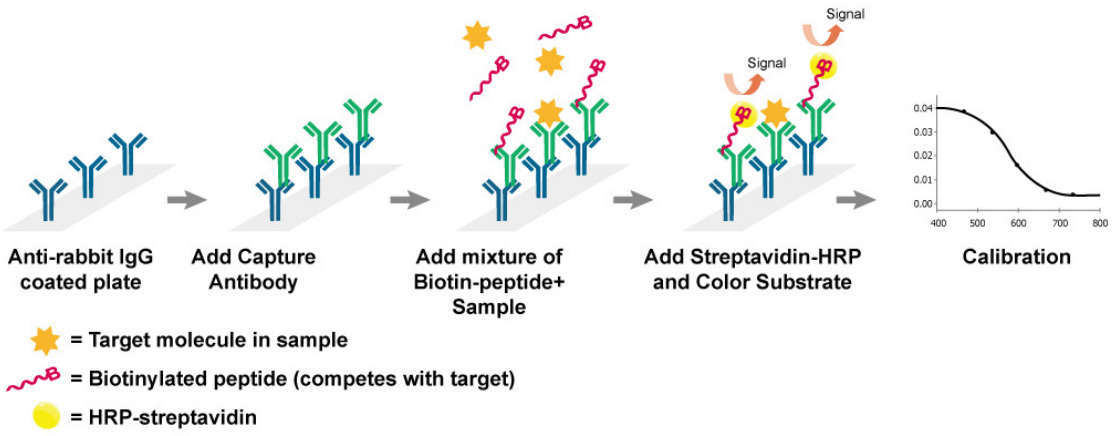
Vaspin ameliorates certain aberrations seen in the diabetic obesity metabolic syndrome by sensitizing insulin action, especially in WATs. Research on Vaspin has been trying for the identification of the potential protease substrate leading to the development of antiprotease inhibitor therapy, which could facilitate the improvement of insulin sensitivity in this metabolic syndrome.

Principle of the Assay

The SERPINA12 (Human/Mouse/Rat) ELISA Kit is an in vitro quantitative assay for detecting Vaspin peptide based on the principle of Competitive Enzyme Immunoassay.

The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-Vaspin antibody, both biotinylated Vaspin peptide and peptide standard or targeted peptide in samples interacts competitively with the Vaspin antibody. Uncompeted (bound) biotinylated Vaspin peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP) which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of Vaspin peptide in the standard or samples. This is due to the competitive binding to Vaspin antibody between biotinylated Vaspin peptide and peptides in standard or samples. A standard curve of known concentration of Vaspin peptide can be established and the concentration of Vaspin peptide in the samples can be calculated accordingly.

Principle of Competitive ELISA



General Information

Materials Supplied

Component	Amount
Vaspin Microplate (Item A) coated with secondary antibody.	96(8x12) wells
Wash Buffer Concentrate (20x) (Item B)	25 ml
Standard Vaspin Peptide (Item C)	10 µl x 2
Anti-Vaspin polyclonal antibody (Item N)	5 µl x 2
Assay Diluent A (Item D): contains 0.09% sodium azide as preservative. Diluent for Standards and serum or plasmas.	30 ml
Assay Diluent B (Item E): 5x concentrated buffer. Diluent for Standards and cell culture media or other sample types.	15 ml
Biotinylated Vaspin peptide (Item F)	20 µl x 2
HRP-Streptavidin concentrate (Item G) 500x concentrated HRP-conjugated Streptavidin.	600 µl
Positive control (Item M)	100 µl
TMB One-Step Substrate Reagent (Item H): 3, 3', 5, 5'-tetramethylbenzidine (TMB) in buffered solution.	12 ml
Stop Solution (Item I): 0.2 M sulfuric acid.	8 ml

Storage Instruction

- Standard, Biotinlated Vaspin peptide, and Positive Control should be stored at -20℃ or -80℃ (recommended at -80℃) after arrival. Avoid repeated freeze-thaw.
- To remaining kit components may be stored at -20℃.
- Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2-8℃. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.
- If stored in this manner, Abnova warranties this kit for 6 months from the date of shipment.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Precision pipettes to deliver 2 µl to 1 ml volumes.
- ✓ Adjustable 1-25 ml pipettes for reagent preparation.
- ✓ 100 ml and 1 liter graduated cylinders.
- ✓ Absorbent paper.
- ✓ Distilled or deionized water.
- ✓ SigmaPlot software (or other softwave which can perform four-parameter logistic regression models)
- ✓ Tubes to prepare standard or sample dilutions.
- ✓ Orbital shaker
- ✓ Aluminum foil
- ✓ Saran Wrap

Assay Protocol

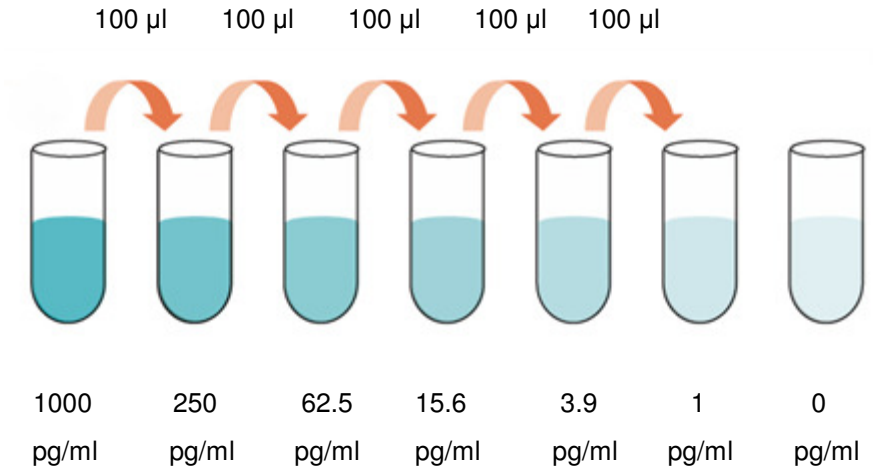
Reagent Preparation

If testing plasma or serum samples, use Assay Diluent A to dilute Item F and Item C. If testing cell culture media or other sample types, use Assay Diluent B to dilute Item F and Item C. For sample and positive control dilutions, refer to step 6, 7, 8 and 10 of Reagent Preparation.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.
2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.
3. Briefly centrifuge the Anti-Vaspin Antibody vial (Item N) before use. Add 50 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.
4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is your anti-Vaspin antibody working solution, which will be used in step 2 of Assay Procedure.

NOTE: the following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure).

5. Briefly centrifuge the vial of Biotinylated Vaspin (Item F) before use. Add 5 µl of Item F to 5 ml of appropriate Assay Diluent. Pipette up and down to mix gently. The final concentration of biotinylated Vaspin will be 100 pg/ml. This solution will be used as diluent in step 6 of Reagent Preparation.
6. Preparation of standard: Label 7 microtubes with the following concentrations: 1000 pg/ml, 250 pg/ml, 62.5 pg/ml, 15.6 pg/ml, 3.9 pg/ml, 1 pg/ml and 0 pg/ml. Pipette 300 µl of biotinylated Vaspin solution in each tube, except for the 1000 pg/ml (leave this one empty). It is very important to make sure the concentration of biotinylated Vaspin is 100 pg/ml in all standards.
    - ✓ Briefly centrifuge the vial of Vaspin (Item C). In the tube labeled 1000 pg/ml, pipette 8 µl of Item C and 792 µl of 100 pg/ml biotinylated Vaspin solution (prepared in step 5). This is your Vaspin stock solution (1000 pg/ml Vaspin, 100 pg/ml biotinylated Vaspin). Mix thoroughly.
    - ✓ To make the 250 pg/ml standard, pipette 100 µl of Vaspin stock solution the tube labeled 250 pg/ml. Mix thoroughly.
    - ✓ Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 300 µl of biotinylated Vaspin and 100 µl of the prior concentration until 1 pg/ml is reached. Mix each tube thoroughly before the next transfer.
    - ✓ The final tube (0 pg/ml Vaspin, 100 pg/ml biotinylated Vaspin) serves as the zero standard (or total binding).



7. Prepare a 10-fold dilution of Item F. To do this, add 2 µl of Item F to 18 µl of the appropriate Assay Diluent. This solution will be used in step 8 and 10.
8. Positive control preparation: briefly centrifuge the positive control vial (Item M). To the tube of Item M, add 101 µl 1x Assay Diluent B. Also add 2 µl of 10-fold dilution of the Item F (prepared in step 7) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample with an expected signal between 10% and 30% of total binding (70-90% competition) if diluted as described above. It may be diluted further if desired, but be sure the final concentration of biotinylated Vaspin is 100 pg/ml.
9. If Item B (20x Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
10. Briefly centrifuge the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 500-fold with 1x Assay Diluent B.
    Note: Do not use Assay Diluent A for HRP-Streptavidin preparation in step 10.

Sample Preparation

Use Assay Diluent A + biotinylated Vaspin to dilute serum/plasma samples. For cell culture medium and other sample types, use 1x Assay Diluent B + biotinylated Vaspin as the diluent. It is very important to make sure the final concentration of the biotinylated Vaspin is 100 pg/ml in every sample.

EXAMPLE: to make a 4-fold dilution of sample, mix together 2.5 µl of 10-fold diluted Item F (Prepared in Reagent Preparation, Step 7) to 185 µl of appropriate Assay Diluent, and 62.5 µl of your sample; mix gently. The total volume is 250 µl, enough for duplicate wells on the microplate.

Do not use Item F diluent from Step 5 (Reagent Preparation) for Sample Preparation. If you plan to use undiluted samples, you must still add biotinylated Vaspin to a final concentration of 100 pg/ml.

EXAMPLE: Add 2.5 µl of 10-fold diluted Item F to 247.5 µl of sample.

NOTE: Optimal sample diluention factors should be determined empirically, however you may contact

technical support to obtain recommended dilution ranges from serum or plasma.

Assay Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of anti-Vaspin antibody (see Reagent Preparation step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec). You may also incubate over night at 4℃.
3. Discard the solution and wash 4 times with 1x Wash Solution (200-300 µl each). Washing may be done with a multichannel pipette or an automated plate washer. 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.
4. Add 100 µl of each standard (see Reagent Preparation step 6), positive control (see Reagent Preparation step 8) and sample (see Sample Preparation) into appropriate wells. Be sure to incubate a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4℃.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of prepared HRP-Streptavidin solution to each well. Incubate for 45 minutes with gentle shaking at room temperature. It is recommended that Incubate time should not be shorter or longer than 45 minutes.
7. Discard the solution and wash 4 times as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl anti-Vaspin antibody to each well. Incubate 1.5 hours at room temperature or overnight at 4℃.
3. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4℃.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit straight line through the standard points.

Percentage absorbance = (B – blank OD)/(B_0 – blank OD) where

B = OD of sample or standard and

B_0 = OD of zero standard (total binding)

Performance Characteristics

- Sensitivity

The minimum detectable concentration of Vaspin is 26.2 pg/ml.

- Linearity

1-10,000 pg/ml

- Reproducibility

Intra-Assay: CV<10%

Inter-Assay: CV<15%

- Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.

Resources

Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
Low signal	Too brief incubation times	Ensure sufficient incubation time; assay procedure step 2 change to over night
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at<-20℃ after reconstitution, others at 4℃. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measure

References

1. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A, Shikata K, Hourai S, Futami J, Watanabe E, Matsuki Y, Hiramatsu R, Akagi S, Makino H, and Kanwar YS (2005). Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci USA. 102(30):10610-5.
2. Wada J (2008). Vaspin: a novel serpin with insulin-sensitizing effects. Expert Opin Investig Drugs 17(3):327-33.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank										
B	Total Binding	Total Binding										
C	Standard 1	Standard 1										
D	Standard 2	Standard 2										
E	Standard 3	Standard 3										
F	Standard 4	Standard 4										
G	Standard 5	Standard 5										
H	Pos Control	Pos Control										