MOUSE VASPIN ELISA KIT

For the quantitative determination of Vaspin concentrations in mouse or rat serum, plasma and cell cultures.

PURCHASE INFORMATION:

ELISA Name	Mouse Vaspin ELISA
Catalog No.	
Lot No.	
Formulation	96 T
Standard range	1.6-1000 ng/mL
Sensitivity	0.097 ng/mL
Sample Volume	50 µl
Dilution Factor	2
Sample Type	Serum, EDTA plasma, cell culture
Specificity	Mouse and Rat
Intra-assay Precision	6-8%
Inter-assay Precision	8-12%
Storage	4 °C

INTRODUCTION

Mouse Vaspin ELISA employs the quantitatively competitive enzyme immunoassay technique in which Mouse Vaspin present in samples competed with a fixed amount of biotinylated Mouse Vaspin for sites on purified rabbit IgG specific against Mouse 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 Mouse Vaspin bound in the initial step. The sample values are then read off the standard curve.

Mouse Vaspin ELSA has been shown to accurately quantitate the recombinant and natural Mouse Vaspin. Results obtained using natural Mouse 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted sulfuric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) for specific advice.

MATERIALS PROVIDED

Description	Code	Quantity
R-Microplate – 96 well microplate precoated with polyclonal anti-rabbit IgG, one plate	RM01	1 plate
Standard – 1000 ng/vial of recombinant Mouse Vaspin in a buffered protein base with preservatives; lyophilized.	560-03-01	1 vial
Biotin Solution-350 µL / vial, 10-fold concentrated of Mouse Vaspin biotinylated with preservatives; lyophilized.	560-03-03	1 vial
Antibody– 350 µL / vial, 10-fold concentrated polyclonal purified IgG against Mouse Vaspin with preservatives; lyophilized.	560-03-02	1 vial
Positive Control – one vial of recombinant Mouse Vaspin , lyophilized (optional)	560-03-04	1 vial
Streptavidin-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer- 60 mL/vial of buffered protein based solution with preservatives	DB06	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with	WB01	1 vial

preservative.		
TMB Substrate Solution-13 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution（0.5M/L sulfuric acid）, 13 ml /vial	S-STOP	1 vial
Plate Covers – Plate sealer.	EAPS	1
Blue Color Indicator Solution- 100 µl/vial, of blue color buffered protein based solution with preservatives	BLUE	1 vial
Yellow Color Indicator Solution- 100 µl/vial, of blue color buffered protein based solution with preservatives	YELLOW	1 vial

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrated should be stored at -20 or -70 °C. Do not use past kit expiration date.

Opened / Reconstituted Reagents: *Reconstituted Standard (1000 ng/ml), Biotin Solution and Antibody SHOULD BE STORED at -20 °C or – 70°C for up to one months. Reconstituted Biotin Solution (350 µl) CAN NOT BE STORED at 2-8°C.* Streptavidin - HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months 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.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 60 µL sample + 60 µL Dilution Buffer. Mix well. Assay immediately

Use polypropylene test tubes.

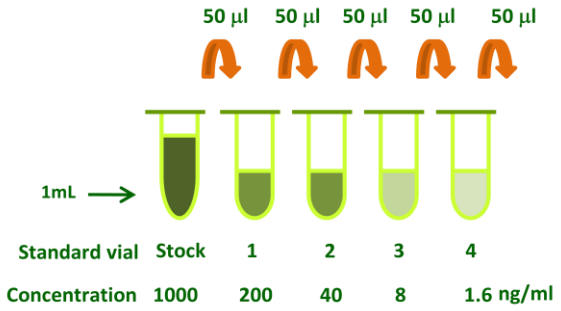
REAGENT PREPARATION

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.

Standard - Refer to vial label for reconstitution volume. Reconstitute the Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 1000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 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 1000 ng/mL standard serves as the high standard.

Standard	Standard	Reagent Diluent	Concentration
stock	powder	1 ml	1000 ng/ml
# 1	50µl of stock	200µl	200 ng/ml
# 2	50µl of 1	200µl	40 ng/ml
# 3	50µl of 2	200µl	8 ng/ml
# 4	50µl of 3	200µl	1.6 ng/ml



Antibody- Reconstitute the **Antibody** with 350µl of Dilution Buffer to make 10 fold concentrated antibody solution. Transfer it into 3.15 mL of Dilution Buffer produce a 1x Antibody solution. Note: Add 5 µL of Blue Color Indicator Solution into 3.5 mL of 1 x Antibody Solution. That indicates 1 x Antibody Solution in the well.

Biotin Solution- Reconstitute the Biotin Solution with 350 µl of **Reagent Diluents** to make 10-fold concentrated solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1 x Biotin Solution. Note: **A**. Add 10 µL of Yellow Color Indicator Solution into 3.5 mL of 1 x Biotin Solution. That indicates 1 x Biotin Solution in the well. **B**. Reconstituted the 10-fold concentrated Biotin Solution or 1 x Biotin Solution SHOULD BE STORED at -20- -70 °C. ***C****. After adding color 1 x Biotin Solution to each well in step 8, the color in the well will change from light blue (antibody solution) to light green.*

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. *Note: 1 x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

Positive Control- Reconstitute the positive control with 1mL of Dilution Buffer to make positive control solution. Note: If mouse Vaspin standard was used as assay standard, the value on the label may change.

ASSAY PROCEDURE

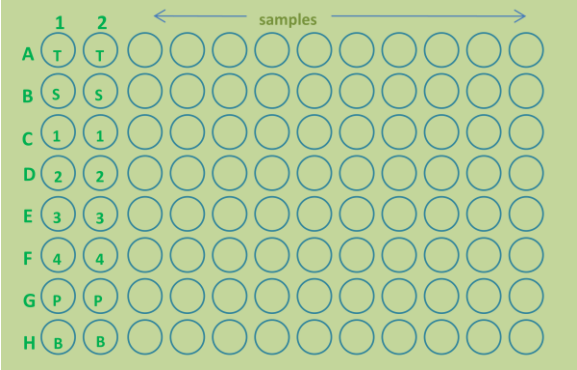
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

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. Leave well H1 and H2 as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELL.***
4. Set A1 and A2 as total binding. Add 50 µl per well of Reagent Diluents.
5. Add 50 µl per well of standard solution from #4 to S (reverse order of serial dilution) to the appropriate wells (B1 to F2). Add 50 µl per well of Positive control into well G1 and G2. Add 50 µl per well of samples into appropriate wells.
6. Add 25µl per well of 1 x Antibody solution into total binding, standard, PC and samples wells.
7. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250-300 rpm). Note: standard, Blank and PC should be assayed in duplicate. ***DO NOT ASPIRATE AND WASH BEFORE ADD BIOTIN SOLUTION.***
8. Add 25 µl per well of 1 x Biotin Solution into total binding, standard, QC and samples wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker.
9. Aspirate wells and wash 5 times with 300 µl of 1 x Assay Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
10. Add 100 µL of **Streptavidin-HRP Conjugate** working solution. Cover or seal the plate and incubate at room temperature for 1 hour on microplate shaker.
11. Repeat the aspiration/wash as in step 9.
12. Add 100 µL of Substrate Solution to each well. Incubate for 20-30 minutes at room temperature. **Protect from light**.
13. 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.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, QC , and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance.

Due samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor 500.



TYPICAL DATA

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

Mouse Vaspin Standard (ng/mL)	Average OD450 (Corrected)
Total Binding	1.223
1.6	1.079
8	0.680
40	0.254
200	0.089
1000	0.018

CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant mouse Vaspin.

SENSITIVITY

The minimum detectable dose (MDD) of Mouse Vaspin Was 0.097 ng/mL.

SPECIFICITY

Mouse Vaspin ELISA kit recognizes recombinant and endogenous mouse Vaspin. The data also indicated that rat 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 rat serum samples cross-react with mouse Vaspin ELISA kit.

Proteins	Cross-reactivity
Mouse Vaspin	100%
Human Vaspin	100%
Mouse FABP-4	0
Mouse Leptin	0
Rat Leptin	0
Mouse gAdiponectin	0
Rat gAdiponectin	0
Mouse FGF21	0
Rat FABP-4	0
Rat Visfatin	0

REFERENCES:

1: Caminos JE, et al. Vaspin and amylin are expressed in human and rat placenta and regulated by nutritional status. Histol Histopathol. 2009 Aug; 24(8):979-90.

2: González CR, et al. Regulation of visceral adipose tissue-derived serine protease inhibitor by nutritional status, metformin, gender and pituitary factors in rat white adipose tissue. J Physiol. 2009 Jul 15;587(Pt 14):3741-50. Epub 2009 May 26.

LINEARITY

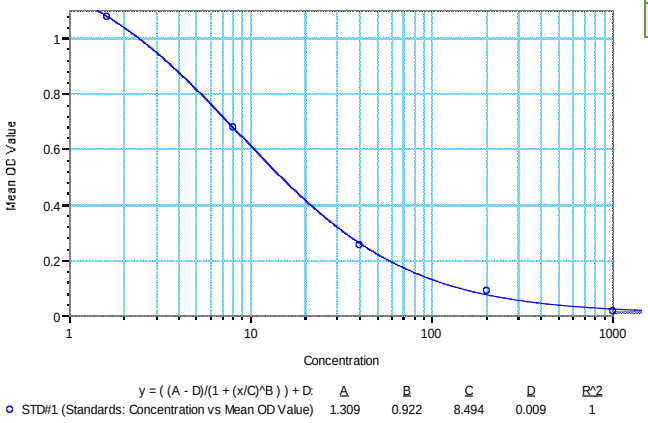
To assess the linearity of the assay, pooled mouse serum samples were diluted with Dilution Buffer BD06 and assayed.

Serum Sample	Conc. (ng/ml)	Final (ng/ml)	Recovery (%)
1 x	16.664		100
0.5 x	9.028	18.056	108

To assess the linearity of the assay, pooled rat EDTA plasma samples were diluted with Dilution Buffer BD06 and assayed.

Plasma Sample	Conc. (ng/ml)	Final (ng/ml)	Recovery (%)
1 x	17.622		100
0.5x	8.483	16.966	96

Mouse Vaspin ELISA Standard Curve, Lot No: 2010018



SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards

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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.

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Add 25 µl Biotin Solution to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Streptatvin HRP conjugate to all wells. Incubate 1 hour on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Substrate to each well. Incubate 20-30min on the bench top. Protect from light.

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Add 100 µl Stop Solution to each well. Read 450nm within 15 min