MOUSE VASPIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF VASPIN CONCENTRATIONS IN MOUSE OR RAT SERUM, PLASMA AND CELL CULTURES

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PURCHASE INFORMATION:

ELISA Name	**MOUSE VASPIN ELISA**
Catalog No.	SK00560-03
Lot No.	
Formulation	96 T
Standard range	1.28 - 800 ng/ml
Sensitivity	0.097 ng/ml
Sample Volume	50 µl
Dilution Factor	2 **(Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum, EDTA plasma, Cell Cultures
Specificity	Mouse and Rat
Calibration	Mouse Vaspin recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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INTRODUCTION

Mouse Vaspin ELISA employs the quantitatively competitive enzyme immunoassay technique in which mouse Vaspin present in samples compete 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 Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of mouse Vaspin bound in the initial step. The sample values are then read off the standard curve.

Mouse Vaspin ELISA has been shown to accurately quantify 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 Dilution Buffer 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 Dilution Buffer and repeat the assay.
_ Any variation in standard buffer, 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 microplate precoated with polyclonal anti-rabbit IgG.	**RM01**	**1 plate**
Vaspin Standard – 4000 ng/vial of recombinant mouse Vaspin in a buffered protein base with preservative; lyophilized.	**560-03-01**	**1 vial**
Antibody Concentrate – 600 µL/vial of 10-fold concentrate polyclonal purified IgG against mouse Vaspin with preservative; lyophilized.	**560-03-02**	**1 vial**
Biotin Concentrate - 600 µL/vial of 10-fold concentrate of mouse Vaspin biotinylated with preservative; lyophilized.	**560-03-03**	**1 vial**
Positive Control – one vial of recombinant mouse Vaspin with preservative; lyophilized (optional).	**560-03-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservative.	**SAHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB18**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrate and Biotin Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: *Reconstituted Standard (4000 ng/ml), Biotin Concentrated Solution and Antibody Concentrated Solution SHOULD BE STORED at -20° C or -70° C for up to one month. Reconstituted Biotin Concentrated Solution (600 µL) CAN NOT BE STORED at 2 – 8° C.* Streptavidin-HRP Conjugate 100-fold concentrated solution and other components may be stored at 2 – 8° C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

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.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Centrifuge and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 65 µL sample + 65 µL Dilution Buffer. Mix well. Assay immediately.
Optimal dilutions should be determined by each laboratory for each application
Use polypropylene test tubes.

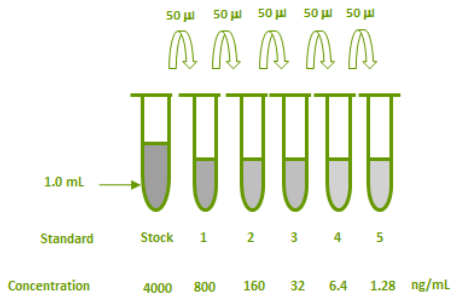
REAGENT PREPARATION

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 1x Wash Buffer.

Vaspin Standard - Reconstitute the Vaspin standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **800 ng/mL** standard serves as the high standard.

Tube	Standard	Dilution Buffer	Concentration
stock	powder	1.0 ml	4000 ng/ml
# 1	50 µl of stock	200 µl	800 ng/ml
# 2	50 µl of 1	200 µl	160 ng/ml
# 3	50 µl of 2	200 µl	32 ng/ml
# 4	50 µl of 3	200 µl	6.4 ng/ml
# 5	50 µl of 4	200 µl	1.28 ng/ml



Positive Control - Reconstitute the Positive Control with 2.0 mL of Dilution Buffer to make positive control solution. **Note:** Positive control solution could be reused within a few days if stored at -20° C or -70° C.

Antibody Concentrate - Reconstitute the Antibody Concentrate with 600 µl of Dilution Buffer to make 10-fold concentrated antibody solution. Transfer it into 5.4 mL of Dilution Buffer to produce 1x Antibody working solution.

Biotin Concentrate - Reconstitute the Biotin Concentrate with 600 µl of Dilution Buffer to make 10-fold concentrated biotin solution. Transfer it to 5.4 mL of Dilution Buffer to prepare 1x Biotin working solution.
Note: 10-fold concentrated Biotin Solution or 1x Biotin working solution SHOULD BE STORED at -20° C or -70° C.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB06)** into a 15 mL centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light)**.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, Total Binding, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the dessicant pack.
3. Leave two wells as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.***
4. Set two wells as total binding. Add 50 µl per well of Dilution Buffer.
5. Add 50 µl per well of standard dilutions from #5 to #S (reverse order of serial dilution) to the appropriate wells. Add 50 µl per well of Positive control solution into another two wells. Add 50 µl per well of samples into other wells.
6. Add 50 µl per well of 1x Antibody working solution into total binding, standard dilutions, Positive control and sample wells.
7. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250-300 rpm). ***DO NOT ASPIRATE AND WASH BEFORE ADDING BIOTIN SOLUTION.***
8. Add 50 µl per well of 1x Biotin working solution into total binding, standard dilutions, Positive control and sample wells.
   Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250-300 rpm).
9. Aspirate wells and wash 5 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
10. Add 100 µL of Streptavidin-HRP Conjugate working solution to all wells, including blanks. Cover with plate sealer and incubate at room temperature for 1 hour on microplate shaker. **Protect from light.**
11. Repeat the aspiration/wash as in step 9.
12. Add 100 µL of Substrate Solution to each well. Incubate for 8-12 minutes at room temperature on microplate shaker. **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 microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for total binding, standard dilutions, Positive control 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.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard (ng/mL)	Average OD450 (Corrected)
Blank	0 (0.091)
Total Binding	1.550
1.28	1.465
6.4	1.299
32	0.704
160	0.243
800	0.085

- Lot No:
- Positive Control:

SPECIFICITY

Mouse Vaspin ELISA kit recognizes recombinant and endogenous mouse Vaspin. Data also indicates that rat serum samples competitively bind 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. This 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

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50 µl of dilution buffer (total binding), standard dilutions, samples, or positive control to each well. Add 50 µl of 1x Antibody working solution to each well used. Incubate 2 hours on the plate shaker at RT. **Do not wash or aspirate before adding Biotin solution**
↓
Add 50 µl 1x Biotin working solution to each well used. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 5 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to all wells, including blanks. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 5 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 8-12 min at room temperature on microplate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.