

MOUSE SOLUBLE FLT-1/VEGF-R1 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE FLT-1/VEGF-R1 CONCENTRATIONS IN SERUM AND PLASMA



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

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	MOUSE SOLUBLE FLT-1/VEGF-R1 ELISA
Catalog No.	SK00114-03
Lot No.	
Formulation	96 T
Standard range	312.5 – 10,000 pg/ml
Sensitivity	70 pg/ml
Sample Volume	100 µL
Sample Type	Serum, Plasma
Dilution factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Mouse sFLT-1
Calibration	Mouse sFLT-1/Fc recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCIERA BIOSCIENCE, INC.
 2348 Walsh Ave., Suite C
 Santa Clara, CA 95051
 USA
 Tel: (408) 982 0300
 Fax: (408) 982 0301
 Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

DESCRIPTION

This Mouse FLT-1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural mouse FLT-1 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant mouse FLT-1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural FLT-1 samples.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for FLT-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FLT-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for FLT-1 is added to the wells. Following a wash to remove any unbound antibody reagent, a Streptavidin-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of FLT-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

PROCEDURAL LIMITATIONS

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

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix 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.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
FLT-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against FLT-1.	114-03-01	1 plate
FLT-1 Standard – refer to lot specific of recombinant mouse FLT-1 in a buffered protein base with preservative; lyophilized.	114-03-02	1 vial
Detection Antibody Concentrate – refer to lot specific, 10-fold concentrate of biotinylated antibody against FLT-1 with preservative; lyophilized.	114-03-03	1 vial
Positive Control - one vial of recombinant mouse FLT-1 in a buffered protein base with preservative; lyophilized.	114-03-04	1 vial
Streptavidin HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin-HRP conjugate.	SAHRP	1 vial
Dilution Buffer - 50 mL of buffered protein based solution with preservative.	DB01	1 bottle
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative;	DB20	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, Detection Antibody Concentrate and lyophilized Antibody Diluent Solution concentrate should be stored at -20° C or -70° C.

For Longer storage for Dilution Buffer (DB01) and Antibody Diluent Solution (DB20), store at -20°C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

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

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.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order code: 00740-01-25, 25 TIU) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Mouse serum or plasma samples may need to be diluted by 2 or 4 fold. A 2-fold dilution is 110 µl of sample + 110 µl of dilution buffer DB01. A 4-fold dilution is 60 µl of sample + 180 µl of dilution buffer DB01.

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.

FLT-1 Standard - Reconstitute the FLT-1 standard with refer to lot specific of **Dilution Buffer (DB01)**. Pipette 250 µl of Dilution Buffer into tubes #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **10,000 pg/ml** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/ml).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	Lot specific	10000 pg/ml
# 1	Lot specific	Lot specific	5000 pg/ml
# 2	250 µl of 1	250 µl	2500 pg/ml
# 3	250 µl of 2	250 µl	1250 pg/ml
# 4	250 µl of 3	250 µl	625 pg/ml
# 5	250 µl of 4	250 µl	312.5 pg/ml
# 6	250 µl of 5	250 µl	156 pg/ml

Positive Control - Reconstitute the positive control with refer to lot specific of **Dilution Buffer (DB01)** to make positive control solution.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to lot specific of **Antibody Diluent Solution (DB20)** to produce a 10-fold concentrated stock solution. Pipette refer to lot specific of Antibody Diluent Solution (DB20) into another 15 mL centrifuge tube and transfer refer to lot specific of 10-fold concentrated stock solution to prepare working solution. **Note: Must be prepared 2 hours prior to use.**

Streptavidin HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 mL of

Dilution Buffer (DB01) to prepare working solution (protect from light).

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, 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. Add 100 µl per well of Dilution Buffer to Blank wells.
3. Add 100 µl of Standard dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for two hours on microplate shaker at room temperature. **Prepare Detection Antibody working solution 2 hours prior to use.**
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. 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.
5. Add 100 µl of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µl of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µl of Substrate Solution to each well. Incubate for refer to lot specific minutes on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

TYPICAL STANDARD CURVE

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

FLT-1 (PG/ML)	CORRECTED (450NM)
Blank	0 (0.140)
156	0.046
312.5	0.082
625	0.162
1250	0.263
2500	0.569
5000	1.229
10000	2.112

SPECIFICITY

PROTEINS	CROSSREACTIVITY (%)
Mouse sFLT-1	100
Human VEGF R1	6.8
Mouse VEGF ₁₆₄	3.9
Rat VEGF ₁₆₄	1.0
Mouse PlGF-2	0
Mouse VEGF-B	0
Mouse VEGF ₁₂₀	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate for 2 hours on plate shaker at RT. Prepare Detection Antibody working solution 2 hours prior to use.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate for 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot specific min on plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read at 450nm.