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Sunlong Medical™ Aflibercept ELISA Kit

Catalog Number: ADA0113

Size: 96 Test

Storage: 2 - 8 °C

Validity Period: 6 months

Sensitivity: 40.12 ng/mL

Assay range: 78.13 - 5,000 ng/mL

Used for the quantitative determination of Aflibercept concentration in serum and plasma.

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

ASSAY PROCEDURE SUMMARY

1. **Prepare all reagents and working standards as directed in the previous sections.**

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2. **Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.**

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3. **Add 100 µL standard or samples to each well.**

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4. **Aspirate each well and wash, repeating the process three times.**

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5. **Add 100 µL of Detection A (working solution) to each well.**

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6. **Repeat the aspiration/wash as in step.**

↓

7. **Add 100 µL of Detection B (working solution) to each well.**

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8. **Aspirate each well and wash, repeating the process five times.**

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9. **Add 100 µL of Color Reagent to each well.**

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10. **Add 50 µL of Stop Solution to each well.**

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11. **Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.**

DESCRIPTION

Used for the quantitative determination of Aflibercept concentration in serum and plasma.

PRINCIPLE OF THE ASSAY

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

MATERIALS PROVIDED (96 Test)

#	Name	Specification	Quantity	Storage
1	Pre-coated Microplate	96 well polystyrene microplate (12 strips of 8 wells) precoated with an antibody specific for Aflibercept	1 plate	-20 ℃
2	Aflibercept Standard	5,000 ng/bottle of lyophilized Aflibercept. Reconstitute in 1 mL Standard Diluent before used	2 bottles	-20 ℃
3	Detection A	120 µL/vial of Biotin labeled antibody specific for Aflibercept (including preservative), 1:100 diluted by Assay Diluent before used	1 vial	-20 ℃
4	Detection B	120 µL/vial of Streptavidin-HRP (including preservative), 1:100 diluted by Assay Diluent before used	1 vial	-20 ℃
5	Standard Diluent	25 mL/bottle diluent (including preservative) was used to dilute the Standard and Samples	1 bottle	2 - 8 ℃
6	Assay Diluent	25 mL/bottle diluent (including preservative) was used to dilute the Detection A and Detection B	1 bottle	2 - 8 ℃
7	20 × Wash Buffer	25 mL/bottle of a 20-fold concentrated solution of buffered surfactant with preservative, 1:20 diluted by deionized water before used	1 bottle	2 - 8 ℃
8	Color Reagent	12 mL/bottle of TMB (Tetramethylbenzidine)	1 bottle	2 - 8 ℃
9	Stop Solution	6 mL/bottle	1 bottle	2 - 8 ℃
10	Plate Sealers	Adhesive strips	4 strips	RT

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 620 nm
- Pipettes and pipette tips
- Deionized or distilled water
- 500 mL graduated cylinder

- Squirt bottle, manifold dispenser, or automated microplate washer
- Test tubes for dilution of standards

SAMPLE COLLECTION AND STORAGE

REAGENT PREPARATION

Wash Buffer

Concentrate - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 25 mL of Wash Buffer Concentrate to 475 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Serum and Plasma

- Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 10 µL of sample + 90 µL of

Standard

Diluent (diluted 1:9). If the sample value is outside the range of the standard curve, the dilution can be adjusted appropriately and the assay can be redetermined. If the antigen concentration in the sample can be estimated and the assay can be performed simultaneously by diluting several gradients prior to the experiment. Standard - Reconstitute with 1 mL Standard Diluent, this reconstitution produces a stock solution of 5,000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. 5,000 ng/mL is the first standard point, and the concentration of the 7 standard sample were 5,000 ng/mL, 2,500 ng/mL, 1,250 ng/mL, 625 ng/mL, 312.5ng/mL, 156.25 ng/mL, 78.13 ng/mL respectively. The appropriate Standard Diluent serves as the zero standard (0 ng/mL). Note： Please use a new standard for each assay and discard after use. Avoid freeze-thaw cycle.

Detection A

(working solution) - Shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the Detection A 1: 100 times to the working concentration with Assay Diluent.

Detection B

(working solution) - Shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the Detection B 1: 100 times to the working concentration with Assay Diluent. Note：Please perform simple centrifugation to collect Detection A and Detection B before use. Prepare Detection A and Detection B immediately before use and avoid diluting the entire stock at once

ASSAY PROCEDURE

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 foil pouch containing the desiccant pack, and reseal.

3. Add 100 µL standard or samples to each well. Cover with the adhesive strip provided. Incubate for 1 hour at 37 ℃.

4. Aspirate each well and wash, repeating the process three times. Wash by filling each well with 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 A (working solution) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37 ℃.

6. Repeat the aspiration/wash as in step.

7. Add 100 µL of Detection B (working solution) to each well. Cover with a new adhesive strip. Incubate for 30 minutes at 37 ℃.

8. Aspirate each well and wash, repeating the process five times. Wash by filling each well with 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.

9. Add 100 µL of Color Reagent to each well. Incubate for 15 minutes at 37 ℃. Protect from light.

10. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm or 620 nm. If wavelength correction is not available, subtract readings at 630 nm or 620 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample. Construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph.

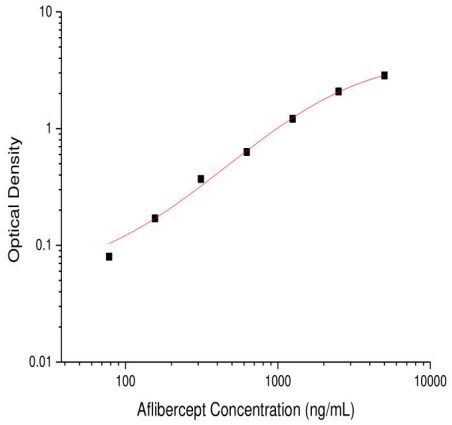
The data may be linearized by plotting the log of the Aflibercept concentrations versus the log of the O.D.

and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

Concentration (ng/mL)	O.D. 1	O.D. 2	Average
0	0.10	0.12	0.11
78.13	0.18	0.08	0.19
0.20	156.25	0.28	0.27
0.17	0.29	0.46	312.5
0.48	0.37	625	0.49
0.75	0.74	0.72	0.63
1250	1.29	1.21	1.32
1.35	2500	2.19	2.14
2.08	2.23	2.93	5000



SENSITIVITY

Sensitivity: the minimum detectable dose (MDD) of Aflibercept is typically less than 40.12 ng/mL.

Detection range: 78.13 - 5,000 ng/mL.

PRECISION

Intra-Assay Precision (Precision within an assay): <10%.

Three samples of known concentration were tested sixteen times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays): <15%.

Three samples of known concentration were tested in twenty four separate assays to assess inter-assay precision.

		Intra-Assay Precision			Inter-Assay Precision		
Sample		1	2	3	1	2	3
n		16	16	16	24	24	24
Mean (ng/mL)		2226.0	599.3	139.1	2254.9	651.9	149.7
Standard deviation		139.0	34.0	13.8	193.3	86.5	21.4
CV (%)		6.2	5.7	9.9	8.6	13.3	14.3

STABILITY

RECOVERY

Sample	Dilution factor	Average (%)	Range (%)
Human serum (n=3)	1:10	94	89-100
	1:20	92	85-107

LINEARITY

Sample	Dilution factor	Average (%)	Range (%)
Human serum (n=3)	1:2	106	104-108
	1:4	112	106-119
	1:8	103	93-116
	1:16	100	92-106

CONTACT US

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