Human Anti-Aflibercept ELISA Kit

Catalog No.: ARE6648 | Size: 48T / 96T | For Research Use Only

1. Product Features

Feature	Description
Application	In vitro quantitative determination of Anti-Aflibercept antibody concentrations in serum, plasma, cell culture supernatant and other biological samples.
Reactivity	Human
Detection Method	Sandwich
Range	0.313-20 ng/mL
Sensitivity	0.188 ng/mL
Detection Duration	3 hours, excluding balancing and sample preparation
Samples needed for single well (Max)	Serum: 50 µL; Plasma: 50 µL; Cell culture supernatant: 100 µL; cell or tissue lysate: 100 µL; other liquid samples: 50 µL.
Specificity	Specifically recognize Anti-Aflibercept antibody, no obvious cross reaction with other analogues.
Storage	2-8°C for sealed box; do not freeze. See kit label for expiry date.

2. Principle of the Assay

This kit is based on sandwich enzyme-linked immunosorbent assay technology. Antigen is pre-coated onto the 96-well plate. The HRP conjugated antigen is used as the detection antigen. The standards and pilot samples are added to the wells subsequently. After incubation, unbound conjugates are removed by wash buffer. Then, HRP detection antigen is added to bind with Anti-Aflibercept antibody conjugated on coated antigen. After washing, TMB substrates are added to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that turns yellow after adding acidic stop solution. Read the O.D. absorbance at 450 nm in a microplate reader. The concentration of target antigen in the sample is positively correlated with OD450 and can be calculated by plotting the standard curve.

3. Kit Components and Storage

The sealed kit can be stored at 2-8°C. The storage condition for opened kit is specified in the table below.

No.	Item	Size (48T)	Size (96T)	Storage Condition for Opened Kit
E001	ELISA Microplate (Dismountable)	8x6	8x12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; stored for 12 months at -20°C.
E002	Lyophilized Standard	1 vial	2 vials	Put the rest standards into a desiccant bag. Stored for 1 month at 2-8°C; stored for 12 months at -20°C.
E003	HRP-labeled Antigen (Concentrated, 100X)	60 µL	120 µL	2-8°C, avoid direct light.
E024	TMB Substrate	5 mL	10 mL	2-8°C, avoid direct light.
E039	Sample Dilution Buffer	10 mL	20 mL	2-8°C.
E040	Antigen Dilution Buffer	5 mL	10 mL	2-8°C.
E026	Stop Solution	5 mL	10 mL	2-8°C.
E038	Wash Buffer (25X)	15 mL	30 mL	2-8°C.
E006	Plate Sealer	3 pieces	5 pieces	2-8°C.
E007	Instruction Manual	1 copy	1 copy	2-8°C.

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label. Please use pipette accurately measure and do proportional dilution.

4. Required Instruments and Reagents

1. Microplate reader (wavelength: 450 nm).
2. 37°C incubator (CO_2 incubator for cell culture is not recommended).
3. Automated plate washer or multi-channel pipette / 5 mL pipettor for manual washing.
4. Precision single and multi-channel pipettes with disposable tips.
5. Sterile tubes, Eppendorf tubes, absorbent paper, loading slot, deionized or distilled water.

5. Sample Collection and Storage

5.1 Serum

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20 min at 1000 x g and collect the supernatant to detect immediately. Or aliquot and store it at -20°C or -80°C for future assay.

5.2 Plasma

EDTA-Na_2/K_2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000 x g, 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately, or aliquot and store it at -20°C or -80°C.

5.3 Tissue Sample

Generally tissue samples are required to be made into homogenization. Place the target tissue on ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01 M, pH 7.4). Use lysate to grind tissue homogenates on ice. Usually, 9 mL PBS would be appropriate to 1 gram tissue pieces. Do further process using ultrasonic disruption or freeze-thaw cycles. Homogenates are then centrifuged for 5 minutes at 5000 x g. Collect the supernatant to detect immediately, or aliquot and store it at -20°C or -80°C.

5.4 Cell Culture Supernatant, Cell Lysate, and Other Biological Samples

For cell culture supernatant, centrifuge at 2500 rpm at 2-8°C for 5 minutes and collect clarified supernatant. For cell lysate, wash cells with pre-cooling PBS, add 0.5-1 mL cell lysate and protease inhibitor, lyse on ice for 30 min-1 h or disrupt by ultrasonic disruption, then centrifuge at 10000 rpm at 2-8°C for 10 minutes. Other biological samples should be centrifuged for 15 minutes at 1000 x g at 2-8°C. Detect immediately or aliquot and store at -80°C.

6. Notes and Precautions

1. Avoid hemolyzed and lipemia samples. Best sample storage: less than 5 days at 2-8°C; within 6 months at -20°C; within 2 years at -80°C.
2. The detection range of this kit is not equivalent to the concentration of analyte in the sample. Properly dilute or concentrate the sample when needed.
3. Sterile and disposable tips are required. Do not reuse tips and tubes to avoid cross contamination.
4. After loading, seal the plate to avoid evaporation during incubation. Wear lab coat, mask and gloves during the assay.

7. Recommended Sample Dilution Ratio

The matrix components in serum/plasma will affect the test results, which need to be diluted at least 1/2 with Sample Dilution Buffer before testing. If another dilution ratio is required, refer to the universal dilution ratio below.

Dilution	Operation
1/2	Add 60 µL sample into 60 µL sample diluent and mix gently.
1/5	Add 24 µL sample into 96 µL sample diluent and mix gently.
1/10	Add 12 µL sample into 108 µL sample diluent and mix gently.
1/20	Add 6 µL sample into 114 µL sample diluent and mix gently.
1/50	Add 3 µL sample and 47 µL normal saline (0.9% NaCl) into 100 µL sample diluent and mix gently.
1/100	Add 3 µL sample and 177 µL normal saline into 120 µL sample diluent and mix gently.
1/1000	Two step dilution. Create a 50-fold dilution first, then create a 20-fold dilution.

Notes: The volume in each dilution is not less than 3 µL. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.

8. Reagent Preparation and Storage

8.1 Wash Buffer: Dilute 30 mL (15 mL for 48T) concentrated wash buffer to 750 mL (375 mL for 48T) wash buffer with deionized or distilled water and mix well. Crystals formed in the concentrated wash buffer can be heated by water bath at 40°C till complete dissolution.

8.2 Standards: Centrifuge standards tube for 1 min at 10000 x g. Add 1 mL sample dilution buffer into the standard tube. Let it stand for 2 min at room temperature and invert several times to mix gently. Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank. Add 0.3 mL sample dilution buffer into each tube. Add 0.3 mL solution from zero tube into 1/2 tube, mix thoroughly, then transfer 0.3 mL stepwise to 1/64 tube. Blank tube only contains sample dilution buffer.

Tube	Concentration
Zero	20 ng/mL
1/2	10 ng/mL
1/4	5 ng/mL
1/8	2.5 ng/mL
1/16	1.25 ng/mL
1/32	0.625 ng/mL
1/64	0.312 ng/mL
Blank	0 ng/mL

8.3 HRP-labeled Antigen Working Solution: Prepare within 30 minutes before the assay. Required total volume: 100 µL/well x quantity of wells, plus additional 100-200 µL. Dilute the HRP detection antigen with antigen dilution buffer at 1:99 and mix thoroughly.

9. Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard or sample	100 µL	90 min	3 times	Seal the plate and incubate at 37°C.
2	HRP-labeled antigen working solution	100 µL	30 min	5 times	Seal the plate and incubate at 37°C.
3	TMB substrate solution	90 µL	10-20 min	Do not wash	Incubate at 37°C in the dark.
4	Stop solution	50 µL	0 min	Do not wash	Read at 450 nm immediately.

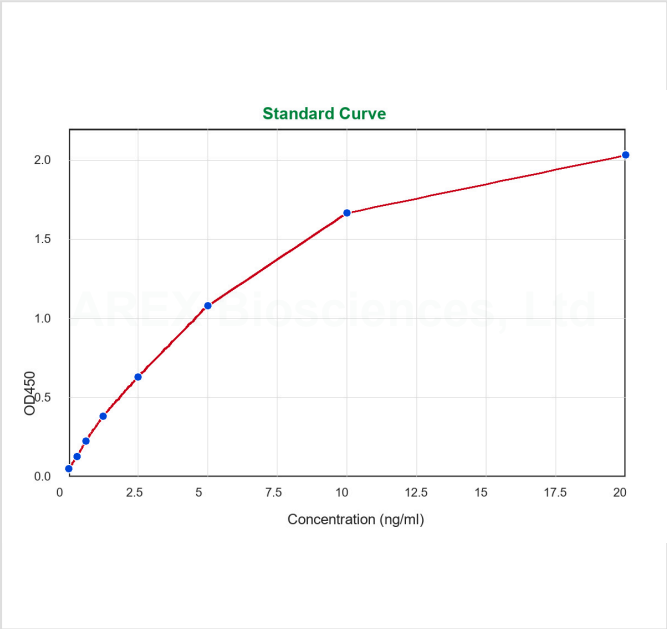
10. Detailed Assay Procedure and Calculation

Set standard, pilot samples, control wells on the pre-coated plate respectively, and record their positions. It is recommended to measure each standard and sample in duplicate. Add standards, blank and samples, incubate for 90 minutes at 37°C, wash three times, add HRP-labeled antigen working solution, incubate for 30 minutes at 37°C, wash five times, add TMB substrate and develop color in the dark, then add stop solution and read OD450 immediately.

Calculate the mean OD450 value of duplicate readings for each standard, control, and sample. Obtain the calculation value by subtracting the OD450 blank. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. Calculate sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

11. Typical Data and Standard Curve

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.



(ng/mL)	O.D	Average	Corrected
0	0.048 0.050	0.049	0
0.312	0.124 0.128	0.126	0.077
0.625	0.223 0.229	0.226	0.177
1.25	0.379 0.389	0.384	0.335
2.5	0.623 0.641	0.632	0.583
5	1.066 1.096	1.081	1.032
10	1.644 1.692	1.668	1.619
20	2.004 2.062	2.033	1.984

12. Performance Characteristics

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	0.66	0.04	5.91
2	20	2.54	0.14	5.42
3	20	10.54	0.63	5.98

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	0.57	0.03	5.29
2	20	2.44	0.13	5.19
3	20	9.86	0.48	4.87

12. Performance Characteristics (continued)

12.1 Recovery

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	86-100	95
EDTA Plasma (n=5)	86-101	94
Heparin Plasma (n=5)	85-105	94

12.2 Linearity

Sample	1:2	1:4	1:8
Serum (n=5)	89-103%	92-101%	89-99%
EDTA Plasma (n=5)	87-103%	85-101%	85-98%
Heparin Plasma (n=5)	85-97%	85-101%	87-99%

12.3 Stability

ELISA kit (n=5)	37°C for 1 month	2-8°C for 6 months	2-8°C for 12 months
Average (%)	80	95-100	85-98

13. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect order for adding reagents; use components from different kits; forget to add reagents.	Confirm required reagent added in each step; use components included in the same kit; repeat the assay and verify.
Overflow OD	Use components from different kits, or prepare working solution with higher concentration.	Use component included in the same kit. Repeat the assay and verify.
Poor standard curve	Inappropriate curve fitting model.	Try to plot the curve by different fitting models.
Samples without signal	Sample is lower than detection range; incompatible buffer; incorrect sample preparation; long storage or freeze-thaw cycle.	Decrease dilution ratio or concentrate sample; verify storage buffer; prepare and store samples according to the assay requirement.
High CV%	Precipitate in well; unclean plate; foam in well; uneven washing; reagents not completely mixed; inconsistent pipetting.	Increase dilution ratio; avoid touching plate bottom; avoid foaming; check washer tube; mix reagents completely; use calibrated pipette.
High Background	Insufficient washing; contaminated wash buffer; too many detection reagents; delayed reading; TMB incubated in strong light.	Follow washing times; use prepared wash buffer immediately; use calibrated pipette; read immediately; incubate in dark.

14. Declaration

This kit is limited to current conditions and scientific techniques. The final assay result is related to the validity of reagents, experimental operation and environment. To get a satisfied assay result, please use all reagents offered by this kit and strictly follow instructions of this manual. This kit allows for research use only.