Mouse VEGF ELISA Kit

Product description

Vascular Endothelial Growth Factor (VEGF) is the most specific regulator of angiogenesis, providing a morphological basis for endothelial cell migration and tumor cell metastasis. As a foundation for tumor stroma and capillary network formation, VEGF can inhibit apoptosis of tumor cells, although the mechanism remains unclear.

The Arcegen Mouse VEGF ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay kit used for quantitative measurement of Mouse VEGF in mouse serum, plasma, and cell culture supernatants. High-affinity anti-VEGF antibodies are precoated on the enzyme-linked immunosorbent assay plate. Standards and test samples are added to the plate wells, and after incubation, VEGF present in the samples binds to the solid-phase antibodies. After washing to remove unbound substances, detection antibodies (biotin-labeled) are added and incubated to bind. After washing, enzyme conjugate (HRP-labeled streptavidin) is added and incubated to bind. Following washing, a colorimetric substrate, TMB, is added for color development in the absence of light. The intensity of the color reaction is directly proportional to the concentration of VEGF in the sample. The reaction is terminated by adding stop solution, and absorbance values are measured at 450 nm as the primary wavelength and 630 nm as the secondary wavelength.

Specifications

Item Number	P162005S / P162005E
Specification	48 T / 96 T
Detection Range	6.25~400 pg/mL
Detection Method	Sandwich ELISA
Species Detected	Mouse
Detection Time	4 hours 30 minutes
Sensitivity	0.11 pg/mL
Dilution Linearity	96.7~126.9%
Recovery Rate	77.1~124%
Intra-assay Variability	4.4%
Inter-assay Variability	6.33%

Components

Component Number	Component Name	Storage Temperature	P162005S	P162005E
P162005-A	ELISA Plate	2~8°C	48 T	96 T
P162005-B	Standard	2~8°C	1 tube	2 tubes
P162005-C	Detection Antibody	2~8°C	120 µL	240 µL
P162005-D	Enzyme Conjugate	2~8°C(Avoid Light)	30 µL	60 µL
P162005-E	5×Dilution Buffer 1	2~8°C	12 mL	25 mL
P162005-F	5×Dilution Buffer 2	2~8°C	8 mL	15 mL
P162005-G	20×Wash Buffer	2~8°C	25 mL	50 mL
P162005-H	Substrate Solution	2~8°C(Avoid Light)	8 mL	15 mL
P162005-I	Stop Solution	Room Temperature	5 mL	10 mL
P162005-J	Plate Sealing Film	Room Temperature	3 pieces	5 pieces

Storage

The kit can be stored at 2~8°C or according to the storage conditions provided for each component to avoid contamination and repeated freeze-thaw cycles. Diluted working concentration reagents should be used immediately and discarded; they should not be reused. The shelf life is 1 year.

Table 1 Reagent Storage Table After Initial Use

Component Name	Storage Conditions
ELISA Plate	Unused strips can be returned to the aluminum foil bag, tightly sealed, and stored at 2~8°C to avoid moisture absorption.
Standard	Use within 48 hours after dissolution, store at 2~8°C to avoid contamination.
Detection Antibody	Use within 48 hours after dilution, store at 2~8°C to avoid contamination.
Enzyme Conjugate	
5×Dilution Buffer 1	Store at 2~8°C for 1 month, avoiding contamination.
5×Dilution Buffer 2	
20×Wash Buffer	
Substrate Solution	Store at 2~8°C for 1 month, avoiding light exposure.
Stop Solution	Can be stored at room temperature.
Plate Sealing Film	

Notes

1. For your safety and health, please wear laboratory coats and disposable gloves when handling.

2. The kit should be used within the shelf life. Mixing related reagents from different batches is prohibited.
3. This product is only intended for detecting target antigens and samples as indicated in the instructions. Other applications should be designed and validated by the user, and the reliability and accuracy should be assessed based on the results.
4. Do not mix or substitute reagents or materials from different kit batches or suppliers.
5. For research use only.

Instructions

1. Used for quantitative detection of Vascular Endothelial Growth Factor (Mouse VEGF) content in serum, plasma, and cell culture supernatants.
2. Please read the instructions carefully before using this product.

Common Technical Tips

1. When the sample OD value is higher than the S1 OD value, further dilution should be carried out in an appropriate sample dilution solution.
2. Avoid foaming during mixing.
3. When adding standard solutions, samples, or other reagents, promptly replace the tips to prevent cross-contamination.
4. Ensure proper sealing of the microplate during incubation or completely cover with a plate sealing film.
5. Completely remove all solutions and buffers during the washing steps.
6. Do not invert the standard solution tube before dissolving. After inverting the standard solution tube, thoroughly mix by gently tapping it after adding the buffer solution, then centrifuge at low speed.
7. Follow the instructions to place the reagents during the experiment.
8. Dispose of buffers promptly after completing the experiment; use and discard immediately.
9. Different product kits have different compositions and should not be cross-used.

Other Preparation Materials

1. ELISA reader, measure absorbance at 450 nm (reference wavelength 630 nm).
2. Incubator, automated microplate washer.
3. Pipettes, 1 μL to 1 mL pipettes with corresponding tips.
4. 100 mL and 1 L graduated cylinders.
5. Standard or sample dilution tubes.
6. Blotting paper.
7. Distilled water or deionized water.

8. Computer and analysis software.

Pre-Experiment Preparation

1. Sample Collection and Processing

1) Cell culture supernatant: Centrifuge at 1,000 × g for 10 minutes to remove debris. Test immediately or aliquot and store at -20°C or below.

2) Serum samples: Collect serum using tubes free from pyrogens and endotoxins. Allow blood to clot for 30 minutes, then centrifuge at 1,000×g for 10 minutes. Test immediately after aliquoting or store at -20°C or below.

3) Plasma samples: Centrifuge at 1,000×g for 30 minutes to collect the sample. Test immediately or aliquot and store at -20°C or below. This assay kit may be suitable for other biological samples. Cell culture supernatant, serum, and plasma have been validated.

【Note】Before testing, visible precipitates in the sample must be removed. Do not use samples with severe hemolysis or high lipid content. Samples should be aliquoted and stored at -20°C to avoid loss of analyte activity. If testing within 24 hours, samples can be stored at 2~8°C to avoid repeated freeze-thaw cycles. Frozen samples should be slowly thawed to room temperature (25°C±3°C) and gently mixed before testing.

If dilution of samples is required, use the specified sample dilution buffer for dilution.

Recommended dilution factors for normal serum samples (for reference only): 20-fold (using 1× Dilution Buffer 1), mix 50 µL of serum sample with 950 µL of 1× Dilution Buffer 1 for a 20-fold dilution;

Recommended dilution factors for normal plasma samples (for reference only): 20-fold (using 1× Dilution Buffer 1), mix 50 µL of plasma sample with 950 µL of 1× Dilution Buffer 1 for a 20-fold dilution;

Recommended dilution factors for cell culture supernatant (for reference only): Undiluted.

Due to differences in the target protein content of samples, the dilution ratio for each sample is recommended based on pre-experimental results or actual conditions.

2. Preparation of the ELISA Plate

The ELISA plate should be brought to room temperature before use.Unused strips should be promptly sealed in a desiccant and stored at 2~8°C, with each sample requiring multiple wells for experimentation.

3. Reagent Preparation

Before use, all reagents and samples need to be equilibrated to room temperature. To ensure experimental accuracy, please complete the equilibration within 15 minutes before use.

1) 1× Wash Buffer Preparation: Equilibrate the concentrate to room temperature and dissolve

thoroughly without crystallization. Mix well and transfer 25 mL of 20×Wash Buffer to distilled water, then bring to a final volume of 500 mL; Specific volumes can be prepared according to the amount needed for each use.

2) 1 × Dilution Buffer 1 Preparation: Equilibrate the concentrate to room temperature and dissolve thoroughly without crystallization. Mix well and transfer 10 mL of 5×Dilution Buffer 1 to distilled water, then bring to a final volume of 50 mL; Specific volumes can be prepared according to the amount needed for each use. 1 × Dilution Buffer 1 is used for the dilution of standard solutions, test serum/plasma samples, detection antibodies, and enzyme conjugates when detecting serum/plasma.

3) 1 × Dilution Buffer 2 Preparation: Equilibrate the concentrate to room temperature and dissolve thoroughly without crystallization. Mix well and transfer 10 mL of 5×Dilution Buffer 2 to distilled water, then bring to a final volume of 50 mL; Specific volumes can be prepared according to the amount needed for each use. 1 × Dilution Buffer 2 is used for the dilution of standard solutions, detection antibodies, and enzyme conjugates when detecting cell culture supernatants.

4) Detection Antibody Preparation: Before use, centrifuge at 10,000 rpm for 20 seconds, then dilute with antibody dilution buffer at a ratio of 1:50 to the working concentration. For example, take 120 μL and dilute with 1×Dilution Buffer to 6 mL; Specific volumes can be prepared according to the amount needed for each use, and mix well.

5) Enzyme Conjugate Preparation: Before use, centrifuge at 10,000 rpm for 20 seconds, then dilute with enzyme dilution buffer at a ratio of 1:200 to the working concentration. For example, take 30 μL and dilute with 1×Dilution Buffer to 6 mL; Specific volumes can be prepared according to the amount needed for each use, and mix well.

6) Preparation of Standard Curve: Prepare 7 sterile 1.5 mL centrifuge tubes, and mark them in sequence according to the standard concentration. Preparation of S1: Take a vial of standard freeze-dried product and dissolve it in the specified amount of 1×Dilution Buffer according to the label, mix thoroughly, marking it as 400 pg/mL. Add 500 μL of 1×Dilution Buffer to each centrifuge tube, first take 500 μL of S1, add it to the first centrifuge tube and mix thoroughly, then take 500 μL to the next marked concentration centrifuge tube and mix thoroughly, prepare a series of 2-fold diluted standard solutions. The initial highest concentration is marked as 400 pg/mL, and the lowest concentration is 6.25 pg/mL, which can be prepared according to the following method. A standard curve needs to be prepared for each test, and standard curves from different assay kits and different times cannot be mixed. For sample testing, 100 μL of standard solution is required for each well, ensuring that the prepared volume is higher than the required volume to avoid insufficient usage.

Table 2 Preparation of Standard Curve System for Vascular Endothelial Growth

Factor (Mouse VEGF) (6.25~400 pg/mL)

Standard Curve	Diluent (μL)	Volume of Standard Added (μL)	Final Concentration of Standard (pg/mL)
S1	As labeled	/	400
S2	500	500	200
S3	500	500	100
S4	500	500	50
S5	500	500	25
S6	500	500	12.5
S7	500	500	6.25
Blank	500	0	0

Operating Instructions

Before use, all reagents and samples need to be equilibrated to room temperature. It is strongly recommended to perform duplicate measurements for all standard samples and test samples.

1. Reagent preparation: Prepare all necessary reagents, dilute the standard samples, and prepare the test samples.
2. Determination of enzyme-linked immunosorbent assay (ELISA) plate: Calculate the number of ELISA plates required for the test samples and standard samples. Remove the ELISA plates from the aluminum foil bag, return the remaining plates to the bag, and seal the bag tightly for storage at low temperature.
3. Soaking of ELISA plates: Add 1×washing solution (350 μL/well) to soak the ELISA plates. After 30 seconds, discard the liquid from the wells and pat dry the ELISA plates. The liquid volume has a significant impact on the test results, so ensure there is no residual washing solution after the final patting.
4. Sample incubation: Add various gradient standard samples and diluted test samples, 100 μL/well. Ensure completion of spotting within 15 minutes and incubate at room temperature for 2 hours.
5. ELISA plate washing: Discard the liquid from the wells, add 1×washing solution (350 μL/well) to wash the plate 3 times, and pat dry the ELISA plates.
6. Antibody incubation: Add the detection antibody, pre-diluted to the working concentration, to the ELISA plates, 100 μL/well, and incubate at room temperature for 2 hours.
7. ELISA plate washing: Discard the liquid from the wells, add 1×washing solution (350 μL/well) to wash the plate 3 times, and pat dry the ELISA plates.
8. Enzyme conjugate incubation: Add the enzyme conjugate, pre-diluted to the working

concentration, to the ELISA plates, 100 μL/well, and incubate at room temperature for 20 minutes.

9. ELISA plate washing: Discard the liquid from the wells, add 1×washing solution (350 μL/well) to wash the plate 3 times, and pat dry the ELISA plates.
10. Color development: Equilibrate the substrate solution to room temperature for 10 minutes before use. Then, add the substrate solution to the ELISA plates, 100 μL/well, and incubate at room temperature in the dark for 10 minutes.
11. Termination: Add 50 μL/well stop solution to the ELISA plates and gently shake the plates to ensure uniform color development.
12. Reading: Read the absorbance values at 450 nm/630 nm within 10 minutes.

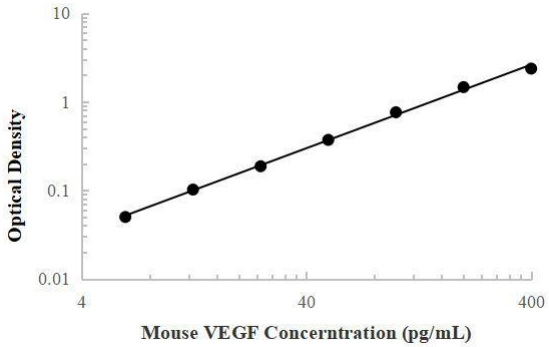
Standard Curve Establishment

Calculate the average OD values of duplicate wells for both standard samples and test samples, and subtract the average OD value of blank wells to obtain the calibrated OD value. Plot the standard curve with the logarithm of the standard sample concentration on the x-axis and the logarithm of the calibrated OD value on the y-axis. Various plotting and statistical software can be used to assist in drawing the standard curve and calculating the concentration of unknown samples. The four-parameter fitting method often yields better fitting results, while other methods such as linear fitting may also yield satisfactory results, depending on the specific experimental data analysis.

Experimental Data

1. Standard Curve Data

Fitting data to generate a standard curve for analysis of experimental data.



Standard Curve Graph

Concentration(pg/mL)	Absorbance		Mean Value	Calibration Value
400	2.461	2.323	2.392	2.365
200	1.476	1.500	1.488	1.462
100	0.769	0.803	0.786	0.760
50	0.417	0.378	0.397	0.371
25	0.217	0.210	0.213	0.187
12.5	0.125	0.131	0.128	0.102
6.25	0.078	0.074	0.076	0.050
0	0.026	0.026	0.026	/

2. Sensitivity Detection

The minimum detection limit of vascular endothelial growth factor is 0.11 pg/mL, calculated by averaging the OD values of 20 repeated measurements of blank wells and determining the standard deviation.

3. Precision Detection

Intra-assay Precision:

The precision within the enzyme-linked immunosorbent assay (ELISA) plate was evaluated by performing 20 replicates of measurements on 3 samples with known concentrations.

Inter-assay Precision:

The precision between ELISA plates was evaluated by performing 36 replicates of measurements on 3 samples with known concentrations.

Project	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
	20	20	20	36	36	36
Mean Value	9.35	41.89	177.21	9.89	42.80	179.60
Standard Deviation	0.36	1.98	8.26	0.81	2.70	8.00
Coefficient of Variation (%)	3.84	4.73	4.66	8.21	6.32	4.45

4. Recovery Rate Detection

The recovery rate was determined by adding different levels of vascular endothelial growth factor to the samples, and the recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	88.8	77.1~103.6
Plasma	89.5	86.4~91.5
Cell culture supernatant	110.0	97.1~124.0

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	107.6	105.3~109.5
1:04	112.1	109.9~115.2
1:08	116.3	110.0~124.2
1:16	122.9	112.2~126.9

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	103.7	102.4~105
1:04	107.6	104.1~111.1
1:08	111.8	111.3~112.4
1:16	122.4	118.5~126.3

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	102.8	100.3~105.3
1:04	104.1	104.0~104.2
1:08	99.4	96.7~102.1
1:16	99.1	98.1~100.1

6. Sample Values

Using this assay kit, several samples were tested with unknown backgrounds.

Sample Types	Sample Number	Mean Value(pg/mL)	Sample Value(pg/mL)
Cell Culture Supernatant	10	n.d.	n.d.
Serum	10	85.20	58~101
Plasma	5	116.80	98~134

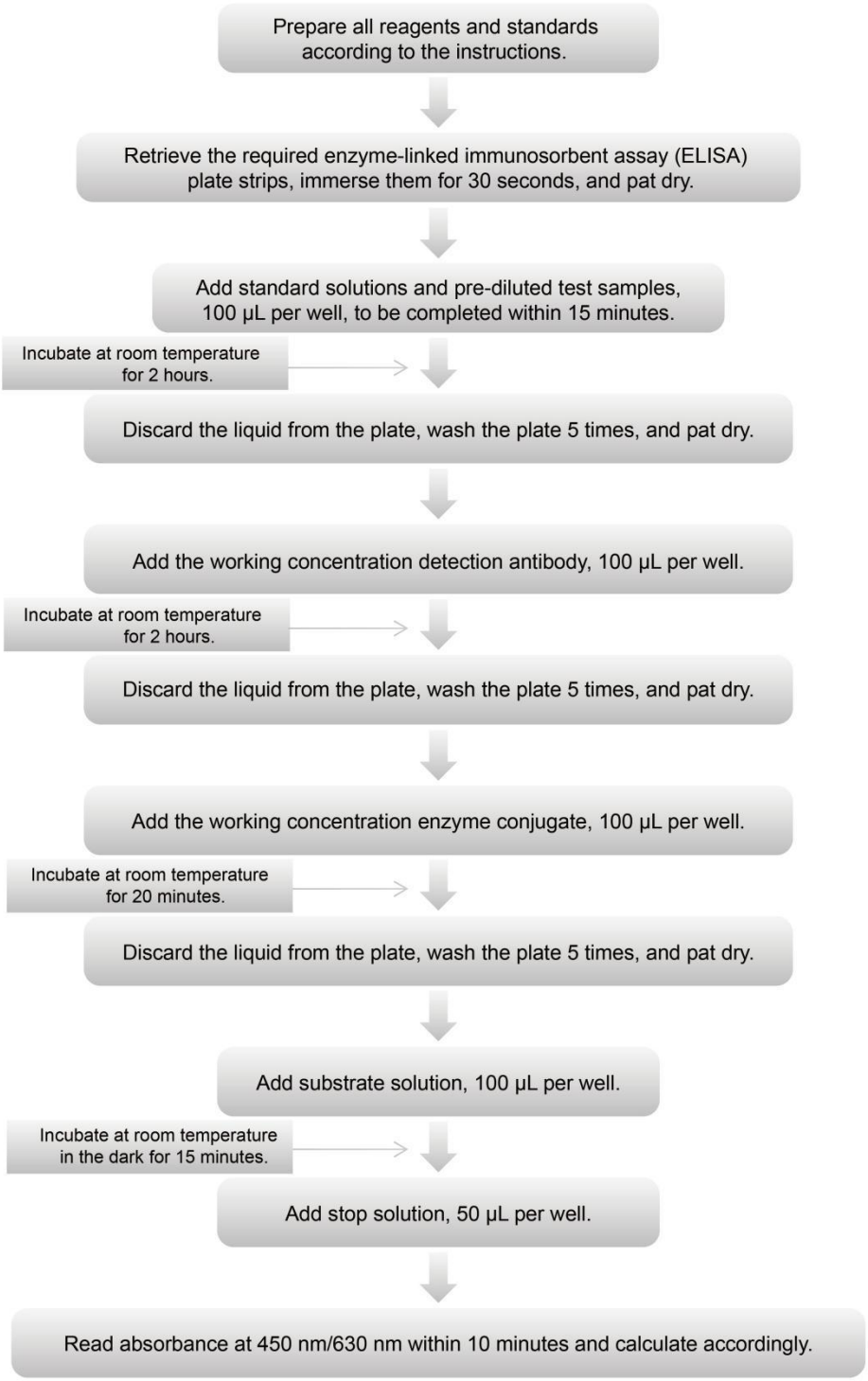
n.d. refers to sample concentrations lower than the detection range of 6.25 pg/mL.

7. Specificity Assay

This assay kit detects both natural and recombinant mouse vascular endothelial growth factor. Specificity evaluation was conducted using a 50 ng/mL preparation of the following factors, and no significant cross-reactivity or interference was observed.

Recombinant mouse:	Recombinant human:	Other Recombinants:
VEGF R2/Fc Chimera	VEGF121	canine VEGF
	VEGF165	feline VEGF
	VEGF165/PIGF	

Detection Schematic



Frequently Asked Questions

Issues	Causes	Solution
Poor standard curve	Inaccurate pipetting volumes	Check the pipette, calibrate it regularly, handle it carefully, tightly cap the tip while mixing thoroughly, and minimize foam formation.
	Inappropriate dilution methods	
Low color intensity	Insufficient incubation time	Allow sufficient incubation time, and replace samples and reconstituted standards overnight.
	Inadequate pipetting volumes or improper dilution	Calibrate pipettes and standardize operations.
High coefficient of variation (CV)	Improper washing of the ELISA plate	Use the correct washing procedure; if using a plate washer, check all ports for blockages.
	Contaminated washing solution	Prepare fresh washing solution.
Low sensitivity	Improper storage of the assay kit	Store according to the product component table.