Basic VEGF-A Human ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human VEGF-A

Catalog Numbers ECH022 (96 tests)

Pub. No. MAN1000740 Rev. A (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Basic VEGF-A Human ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human VEGF-A. This assay is designed to detect and quantify the level of human VEGF-A in serum, plasma (EDTA, citrate, and heparin), and cell culture supernatant.

Vascular Endothelial Growth Factor (VEGF) is a key regulator of angiogenesis, produced by various cell types, including endothelial cells, macrophages, and tumor cells. VEGF promotes the growth and permeability of blood vessels by binding to its receptors, VEGFR-1 and VEGFR-2. Dysregulated VEGF signaling is implicated in various pathological conditions, including cancer, diabetic retinopathy, and age-related macular degeneration. VEGF's role in promoting blood vessel formation is crucial for normal development and wound healing. However, its overexpression can lead to pathological angiogenesis, contributing to tumor growth and ocular diseases. Therapeutic inhibition of VEGF signaling pathways has been successful in treating cancers and ocular disorders characterized by abnormal blood vessel growth.

Contents and storage

- Store kit reagents at 2–8°C.
- Immediately after use, return remaining reagents to cold storage (2–8°C).
- See the expiration date on the package.
- The kit components' expiry is guaranteed only if they are stored properly and not contaminated during repeated use.
- Do not mix components from other lots.

Components	Amount
Coated Microwell Strips	1 pouch (12 strips with 8 wells each)
Biotin-Conjugate (100X)	120 µL
Streptavidin-HRP (100X)	150 µL
Human VEGF-A Standard, lyophilized (2 ng/mL upon reconstitution)	2 vials
Sample Diluent	12 mL
Assay Buffer Concentrate 20X	5 mL
Wash Buffer Concentrate 20X	50 mL
Substrate Solution (Tetramethyl-benzidine)	15 mL
Stop Solution (1M Phosphoric acid)	15 mL
Adhesive Film	6

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm (620 nm as optional reference wavelength)
- Beakers, flasks, and cylinders for preparation of reagents
- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Statistical calculator with program to perform regression analysis
- Microplate shaker

Before you begin

- Equilibrate the buffer concentrates to room temperature (18–25°C), then dilute before use.
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.

Prepare Wash Buffer (1X)

1. Transfer the entire contents (50 mL) of the Wash Buffer Concentrate (20X) to a clean 1,000-mL graduated cylinder, then add 950 mL of glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle, then label as 1X Wash Buffer.
3. Store Wash Buffer (1X) at 2–25°C for up to 30 days.

Prepare Assay Buffer (1X)

1. Transfer the entire contents (5 mL) of the Assay Buffer Concentrate (20X) to a clean 100-mL graduated cylinder, then add 95 mL of distilled water. Mix gently to avoid foaming.
2. Label as 1X Assay Buffer.
3. Store Assay Buffer (1X) at 2–8°C for up to 30 days.

Prepare 1X Biotin-Conjugate

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1X) in a clean plastic tube.

IMPORTANT! Prepare Biotin-Conjugate within 30 minutes of usage.

Dilute 0.12 mL of Biotin-Conjugate (100X) with 11.88 mL of Assay Buffer (1X), then mix thoroughly.

Prepare 1X Streptavidin-HRP

Make a 1:100 dilution of the concentrated Streptavidin-HRP Conjugate in a clean plastic tube.

IMPORTANT! Prepare 1X Streptavidin-HRP within 30 minutes of usage.

Dilute 0.12 mL of concentrated Streptavidin-HRP conjugate with 11.88 mL of Assay Buffer (1X), then mix thoroughly.

Prepare Human VEGF-A Standard

Prepare fresh standard on each day of use as it cannot be stored.

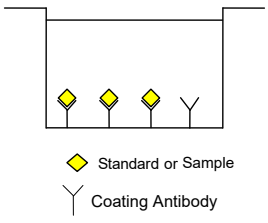
1. Reconstitute human VEGF-A standard by addition of distilled water. The reconstitution volume is stated on the label.

   Note: The concentration of the reconstituted standard is 2 ng/mL.
2. Swirl or mix gently to ensure complete and homogeneous solubilization
3. Before making dilutions, allow the standard to reconstitute for 10–30 minutes, then mix well.
4. Proceed to prepare standard dilutions on a microwell plate.

Perform ELISA protocol

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Shaking is absolutely necessary for optimal test performance.

1 Bind antigen



1. Wash the microwell strips twice with approximately 400 µL of 1X Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Do not scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively, microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
2. **Prepare Human VEGF-A Standard dilutions on the microwell plate as follows:**
   a. Add 100 µL of Sample Diluent, in duplicate, to all standard wells.
   b. Add 100 µL of the reconstituted standard (concentration = 2000 pg/mL), in duplicate, to wells A1 and A2.

1 *(continued)*

c. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 1000 pg/mL), then transfer 100 µL to wells B1 and B2, respectively. Do not scratch the inner surface of the microwells.

d. Repeat the above procedure 5 times, creating two rows of Human VEGF-A Standard dilutions ranging from 1000 pg/mL to 15.6 pg/mL. Discard 100 µL of the contents from the last microwell (G1/G2=S7) used.

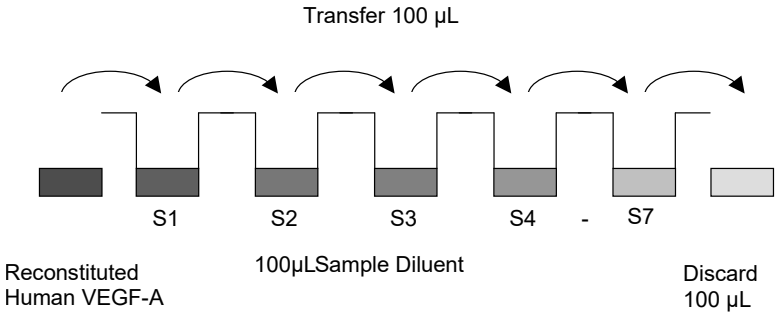


Figure 1 Standard dilutions on the microwell plate

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips

	1	2	3	4	5	6	7	8	9	10	11	12
	Standard		Sample									
A	1	1	1	1	9	9	17	17	25	25	33	33
B	2	2	2	2	10	10	18	18	26	26	34	34
C	3	3	3	3	11	11	19	19	27	27	35	35
D	4	4	4	4	12	12	20	20	28	28	36	36
E	5	5	5	5	13	13	21	21	29	29	37	37
F	6	6	6	6	14	14	22	22	30	30	38	38
G	7	7	7	7	15	15	23	23	31	31	39	39
H	Blank	Blank	8	8	16	16	24	24	32	32	40	40

3. Add 100 µL of Sample Diluent in duplicate to the blank wells.

4. Add 50 µL of Sample Diluent in duplicate to the sample wells.

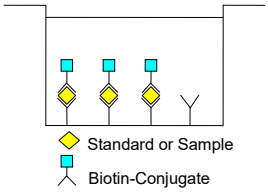
5. Add 50 µL of sample in duplicate to the sample wells.

6. Cover the plate with adhesive film and incubate 2 hours at room temperature on a microplate shaker set at 400 rpm.

7. Prepare 1X Biotin-Conjugate as mentioned in “Prepare 1X Biotin-Conjugate” on page 2.

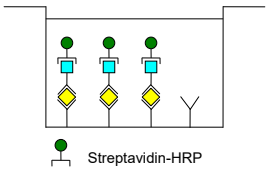
8. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 6 times with 1X Wash Buffer. Allow Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

2 Add 1X Biotin Conjugate



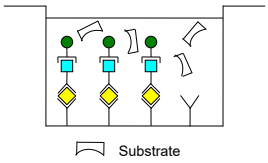
1. Add 100 µL of 1X Biotin-Conjugate to all wells.
2. Cover the plate with an adhesive film and incubate for 1 hour at room temperature on a microplate shaker set at 400 rpm.
3. Prepare 1X Streptavidin-HRP as mentioned in “Prepare 1X Streptavidin-HRP” on page 2.
4. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 6 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

3 Add 1X Streptavidin-HRP Conjugate solution



1. Add 100 µL of 1X Streptavidin-HRP Conjugate to all wells, including the blank wells.
2. Cover the plate with an adhesive film and incubate for 1 hour at room temperature on a microplate shaker set at 400 rpm.
3. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 6 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

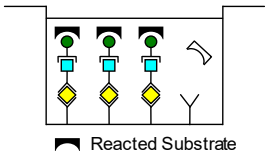
4 Add TMB Substrate Solution



1. Add 100 µL of TMB Substrate Solution to all wells.
2. Incubate the microwell strips at room temperature for about 30 minutes. Avoid direct exposure to intense light.

   Note: The color development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

5 Add Stop Solution



It is recommended to add the stop solution when the highest standard develops a dark blue color.

Add 100 µL of Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

IMPORTANT! It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme.

Calculation of results

Read the absorbance on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable as well). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the controls.

IMPORTANT! Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2–8°C in the dark.

Note: If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (×2).

A representative standard curve is shown in Figure 2.

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Human VEGF-A was diluted in serial 2-fold steps in Sample Diluent.

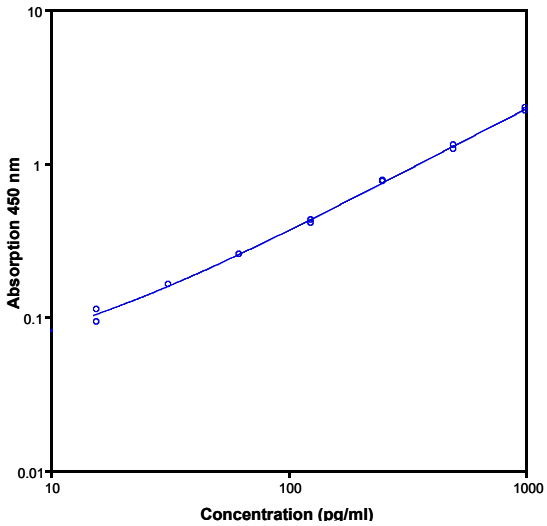


Figure 2 Representative standard curve for human VEGF-A ELISA

Table 2 Typical data using the human VEGF-A ELISA (measuring wavelength of 450 nm, reference wavelength of 620 nm)

Standard	Human VEGF-A Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	1000.0	2.201 2.308	2.254	2.4
2	500.0	1.244 1.327	1.286	3.2
3	250.0	0.766 0.775	0.771	0.6
4	125.0	0.409 0.429	0.419	2.5
5	62.5	0.258 0.258	0.258	0.1
6	31.3	0.162 0.163	0.163	0.3
7	15.6	0.112 0.093	0.102	9.4
Blank	0	0.066 0.073	0.069	4.9

The OD values of the standard curve may vary according to the conditions of assay performance (for example, operator, pipetting technique, washing technique, or temperature effects).

Performance characteristics

Sensitivity

The limit of detection of human VEGF-A defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 7.9 pg/mL (mean of 6 independent assays).

Specificity

The assay detects both natural and recombinant human VEGF-A. Cross-reactivity and interference of circulating factors of the immune system were evaluated by spiking these proteins at physiologically relevant concentrations into a human VEGF-A positive serum. No cross-reactivity was detected, notably not with human VEGF-B, VEGF-C, VEGF-D and PfGF. Interference was detected for VEGF-R1 at concentrations >200 pg/mL, and not for VEGF-R2.

Expected values

Panels of 40 serum as well as EDTA, citrate, and heparin plasma samples from randomly selected apparently healthy donors were tested for human VEGF-A. The levels measured may vary with the sample collection used.

Sample matrix	Number of samples evaluated	Range (pg/mL)	Detectable (%)	Mean of detectable (pg/mL)
Serum	40	nd [1]–42.6	2.5	–
Plasma (EDTA)	40	nd–128.9	7.5	45.7
Plasma (citrate)	40	nd–66.2	7.5	47.3
Plasma (heparin)	40	nd–311.4	7.5	144.3

[1] nd = nondetectable, samples measured below the lowest standard point are considered to be nondetectable.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000740 A (30)

Revision	Date	Description
A (30)	7 December 2024	New document for Basic VEGF-A Human ELISA Kit.

The information in this guide is subject to change without notice.