Performance characteristics, continued

Intra-assay precision

Samples with known VEGF concentration were assayed in replicates of 14 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	87.4	345	938
SD	4.8	12.7	45.8
%CV	5.5	3.7	4.9

SD = Standard Deviation; CV = Coefficient of Variation

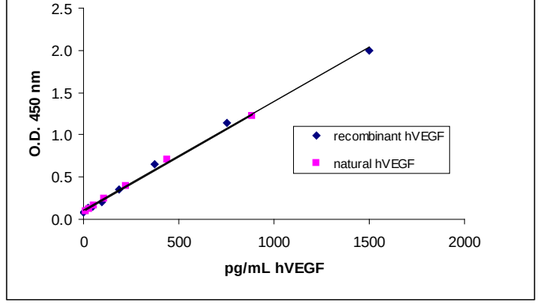
Recovery

The following table shows the average recovery when adding Hu VEGF to the listed sample types.

Sample type	Average % Recovery
Serum	95
Citrate plasma	99
Culture medium containing 1% fetal bovine serum	90
culture medium containing 10% fetal bovine serum	88

Parallelism

Natural Hu VEGF was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. Parallelism between the natural and recombinant protein is demonstrated by the figure below and indicated that the standard accurately reflects natural Hu VEGF content in samples.



Inter-assay precision

Samples were assayed 42 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	88.6	319	984
SD	8.2	27.1	64.4
%CV	9.3	8.5	6.5

SD = Standard Deviation; CV = Coefficient of Variation

Linearity of dilution

Human serum and tissue culture medium containing 10% fetal bovine serum were spiked with Hu VEGF and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

	Serum			Cell Lysate		
Dilution	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
Neat	1834	—	—	1497	—	—
1/2	893	917	97	745	749	99
1/4	493	459	107	386	374	103
1/8	245	229	107	196	187	105
1/16	118	115	103	93	94	99
1/32	58	57	102	47	47	100

Expected values

Human bronchial smooth muscle cells were cultured for 144 hours with and without Hu TNF-α (10 ng/mL). The values were 790 pg/mL and 630 pg/mL, respectively.

Fifteen fresh matched sera from healthy individuals were evaluated in this assay. The values for sera ranged from 40–600 pg/mL (mean 270 pg/mL).

Thirteen previously frozen citrate plasma samples were evaluated in this assay. All samples fell below the sensitivity of the assay

Important licensing information

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

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10 March 2015

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Human VEGF ELISA Kit

Catalog nos. KHG0111 — **Quantity:** 96 tests

KHG0112 — 192 tests

Pub. No. MAN0003960 **Rev** 2.0

Description

The human VEGF ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is be used for the *in vitro* quantitative determination of Human Vascular Endothelial Growth Factor (Hu VEGF) in human serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Hu VEGF–165.

Vascular Endothelial Growth Factor (VEGF), originally named vascular permeability factor (VPF), is an important regulator of angiogenesis and vasculogenesis. Angiogenesis occurs in normal processes related to the female reproductive cycle as well as pathological processes. Vasculogenesis involves the formation of blood vessels through the differentiation of endothelial cells from mesodermal precursors. Whereas vasculogenesis is restricted to embryonic development, angiogenesis operates throughout life when new vascularization is required.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	96 Test Kit	192 Test Kit
Hu VEGF Standard (recombinant Hu VEGF-165 expressed in HEK 293 cells cells), contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer, contains 0.1% sodium azide	25 mL	2 × 25 mL
Incubation Buffer	12 mL	12 mL
Antibody Coated Wells, 96 Wells per plate	1 plate	2 plates
Hu VEGF Biotin Conjugate, (Biotin-labeled anti-VEGF), contains 0.1% sodium azide	11 mL	2 × 11 mL
Streptavidin-HRP (100X), contains 3.3 mM thymol	0.125 mL	2 × 0.125 mL
Streptavidin HRP Diluent, contains 3.3 mM thymol	25 mL	25 mL
Wash Buffer Concentrate (25X)	100 mL	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL	25 mL
Stop Solution	25 mL	25 mL
Adhesive Plate Covers	3	6

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **www.lifetechnologies.com/manuals** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

For Research Use Only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.
3. Store the concentrate and the Working Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Dilute samples

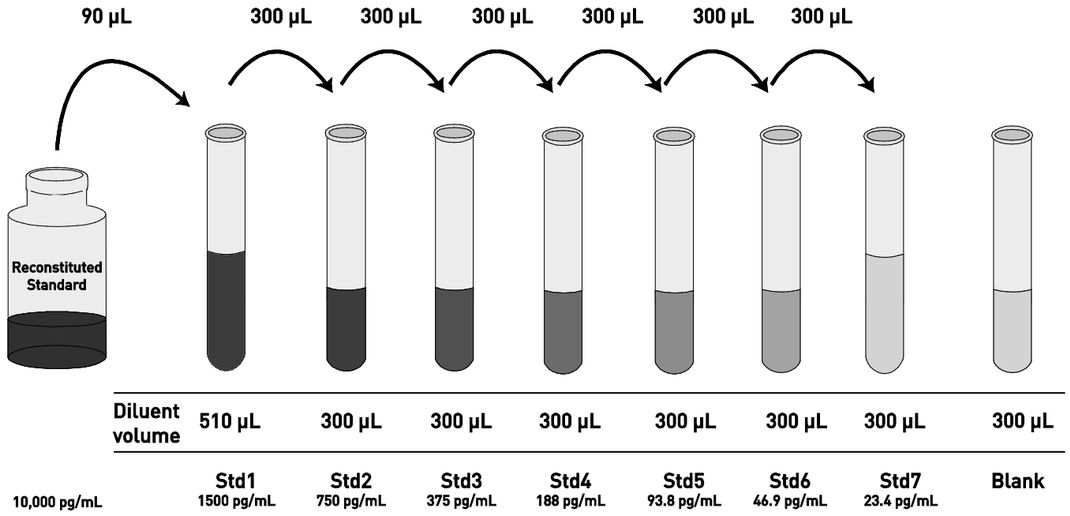
- Dilute **serum and plasma** samples 2-fold in Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu VEGF Standard to 10,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution.
2. Add 90 µL of the reconstituted standard to a tube containing 510 µL Standard Diluent Buffer. Label as 1500 pg/mL Hu VEGF. Use the standard within 1 hour of reconstitution.
3. Add 300 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 750, 375, 188, 93.8, 46.9, and 23.4 pg/mL of Hu VEGF.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.

Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare Streptavidin-HRP solution

Note: Prepare Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

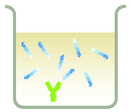
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 990 µL of HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3 hours.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



Bind antigen

1. Add 50 µL of the Incubation Buffer to all wells except chromogen blanks.
2. Add 100 µL of standards to the appropriate microtiter wells.
3. For samples and controls, add 50 µL of Standard Diluent Buffer to each appropriate well followed by 50 µL of sample or controls.
4. Cover the plate with plate cover and incubate for 2 hours at room temperature.
5. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.

Add detector antibody

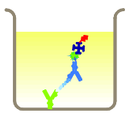
6. Add 100 µL Hu VEGF Biotin Conjugate solution into each well except chromogen blanks.
7. Cover the plate with plate cover and incubate for 1 hour at room temperature.
8. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.

Add Streptavidin-HRP

9. Add 100 µL Streptavidin-HRP (see page 2) into each well except the chromogen blanks.
10. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
11. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

Add chromogen

12. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
13. Cover the plate with plate cover and incubate for 30 minutes at room temperature **in the dark**.
    Note: TMB should not touch aluminum foil or other metals.



Add stop solution

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

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–1500 pg/mL Hu VEGF.

Standard Hu VEGF (pg/mL)	Optical Density (450 nm)
1500	2.63
750	1.62
375	0.86
188	0.48
93.8	0.25
46.9	0.15
23.4	0.11
0	0.06

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the human VEGF kit. The following substances were tested and found to have no cross–reactivity: human IL-1β, IL-2, IL-6, IL-8, IL-10, IL-13, IL-15, EGF, FGF basic, FGF acidic, G-CSF, GM-CSF, IFN-γ, RANTES, SCF, TGF-α, and TNF-α; mouse IL-1β, IL-6, IL-10, G-CSF, GM-CSF, IFN-γ, and TNF-α; rat IL-1β, IL-6, IL-10, GM-CSF, IFN-γ, and TNF-α. Mouse and rat VEGF-165 showed 0.25% and 0.11% cross–reactivity, respectively. Human VEGF-121 showed 100% cross–reactivity and complete parallelism with Hu VEGF-165.

Sensitivity

The minimum detectable concentration of VEGF is <5 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.