OriGene Technologies Inc.
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Human VEGF-C ELISA Kit

Catalog Number: EA100404

Assay Principle

OriGene's human VEGF-C ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for VEGF-C has been precoated onto 96-well plates. Standards (NSO, T103-R227) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for VEGF-C is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added, and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human VEGF-C amount of sample captured in plate.

Overview

Product Name	Human VEGF-C ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human VEGFC in cell culture supernatants, cell lysates, serum, and plasma (heparin, EDTA). 96wells/kit, with removable strips.
Sensitivity	<3 pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	62.5 pg/ml – 4000 pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
Uniprot ID	P49767

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Technical Details

Capture/Detection Antibodies	*The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.*
Specificity	*Natural and recombinant Human VEGFC*
Immunogen	*Expression system for standard: NSO; Immunogen Sequence: T103-R227*
Cross Reactivity	*There is no detectable cross-reactivity with other relevant proteins.*

Notice Before Application

Please read the following instructions before starting the experiment.

1. *To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards, and a small number of samples is recommended.*
2. *Before using the Kit, spin tubes and bring down all components to the bottom of tubes.*
3. *Don't let 96-well plate dry, for dry plate will inactivate active components on plate.*
4. *Don't reuse tips and tubes to avoid cross contamination.*
5. *Avoid using the reagents from different batches together.*

Kit Components/Materials Provided

Description	Quantity	Volume	Storage of opened/reconstituted material
Anti-Human VEGFC Pre-coated 96-well strip microplate	**1**	**12 strips of 8 wells**	**Return unused wells to the foil pouch. Reseal along the entire edge of the zip seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Human VEGFC Standard	**2**	**20 ng/tube**	**Discard the VEGFC stock solution after 12 hours at 4°C. May be stored at -20°C for 49 hours.**
Human VEGFC Biotinylated antibody (100x)	**1**	**100 µl**	**May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Avidin-Biotin-Peroxidase Complex (100x)	**1**	**100 µl**	
Sample Diluent	**1**	**30ml**	
Antibody Diluent	**1**	**12ml**	
Avidin-Biotin-Peroxidase Diluent	**1**	**12ml**	
Color Developing Reagent (TMB)	**1**	**10ml**	
Stop Solution	**1**	**10ml**	
Wash Buffer (25x)	**1**	**20ml**	
Plate Sealers	**4**	**Piece**	

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Required Materials That Are Not Supplied

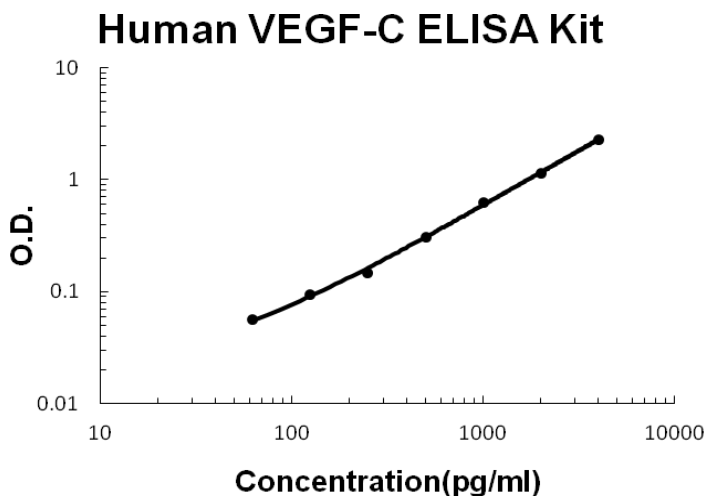
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Typical Human VEGF-C ELISA Kit (EA100404) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	62.5	125	250	500	1000	2000	4000
O.D.	0.110	0.175	0.217	0.298	0.482	0.792	1.413	2.057

Human VEGF-C *ELISA Kit standard curve*



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): *Three samples of known concentration were tested on one plate to assess intra-assay precision.*
Inter-Assay Precision (Precision across assays): *Three samples of known concentration were tested in separate assays to assess inter-assay precision.*

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	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	86	845	1351	89	836	1341
Standard deviation	4.04	56.61	72.95	5.51	71.89	76.43
CV(%)	4.7 %	6.7 %	5.4 %	6.2 %	8.6 %	5.7 %

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	86	84	90	88	87	2.23	2.5%
Sample 2	845	745	816	748	788	43.24	5.4%
Sample 3	1351	1280	1349	1264	1311	39.41	3%

*number of samples for each test n=16.

Preparation Before the Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. Please do not equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25min) is based on 37°C.
Wash buffer	Prepare 500ml of Working Buffer by diluting the supplied 20ml of Wash Buffer (25x) with 480ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-Human VEGFC antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human VEGFC Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody(100x) to 99µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human VEGFC Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the

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	experiment. Use one 20 ng of lyophilized Human VEGFC standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 20 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	*The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.*

Dilution of Human VEGFC Standard

1. *Number tubes 1-8. Final Concentrations to be Tube #1 –4,000 pg/ml, #2 – 2,000 pg/ml, #3 –1,000 pg/ml, #4 –500 pg/ml, #5 – 250 pg/ml, #6 – 125 pg/ml, #7 – 62.50 pg/ml, #8 – 0.0 (Blank – Sample diluent serves as the zero standard).*
2. *To generate standard #1, add 400 µl of the reconstituted standard stock solution of 10 ng/ml and 600 µl of sample diluent to tube #1 for a final volume of 1000 µl. Mix thoroughly.*
3. *Add 300 µl of sample diluent to tubes # 2-7.*
4. *To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.*
5. *To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.*
6. *Continue the serial dilution for tube #4-7.*
7. *Tube #8 is a blank standard to be used with every experiment.*

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	*Procedure*
Cell culture supernatants	*Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.*
Serum	*Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.*
Plasma	*Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.* *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.*
Cell lysate	*Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10,000 x g for 5 min. Collect the supernatant. Assay immediately or store samples at-20°C.*

Sample Dilution

The target protein concentration should be estimated, and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

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Assay Protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before the Experiment if you have missed this information).

1. *Prepare all reagents and working standards as directed previously.*
2. *Remove excess microplate strips from the plate frame and seal and store them in the original packaging.*
3. *Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control are recommended.*
4. *Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).*
5. *Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
6. *Add 100 µl of the prepared 1x Biotinylated Anti-Human VEGFC antibody to each well.*
7. *Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).*
8. *Wash the plate 3 times with the 1x wash buffer.*
   a. *Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
   b. *Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).*
   c. *Repeat steps a-b 2 additional times.*
9. *Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).*
10. *Wash the plate 5 times with the 1x wash buffer.*
    a. *Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
    b. *Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).*
    c. *Repeat steps a-b 4 additional times.*
11. *Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)*
12. *Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.*
13. *Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.*

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

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Background on VEGFC

Vascular endothelial growth factor C is a VEGF. The human gene encoding it is VEGFC. The protein encoded by this gene is a member of the platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF) family, is active in angiogenesis, lymphangiogenesis and endothelial cell growth and survival, and can also affect the permeability of blood vessels. This secreted protein undergoes a complex proteolytic maturation, generating multiple processed forms which bind and activate VEGFR-3 receptors. Only the fully processed form can bind and activate VEGFR-2 receptors. This protein is structurally and functionally similar to vascular endothelial growth factor D (VEGF-D). The C terminus of VEGFC has cysteine-rich repeat units characteristic of the Balbiani ring 3 protein (BR3P) of the midge Chironomus tentans.[1,2] The standard product used in this kit is recombinant human VEGF-C, consisting of 125 amino acids with the molecular mass of 23Kda after glycosylation

Reference

1. *Joukov, V.; Pajusola, K.; Kaipainen, A.; Chilov, D.; Lantinen, I.; Kukk, E.; Saksela, O.; Kalkkinen, N.; Alitalo, K. : A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. EMBO J. 15: 290-298, 1996.*
2. *Lee, J.; Gray, A.; Yuan, J.; Luoh, S.-M.; Avraham, H.; Wood, W. I. : Vascular endothelial growth factor- related protein: a ligand and specific activator of the tyrosine kinase receptor Flt4. Proc. Nat. Acad. Sci. 93: 1988-1992, 1996.*