Chondrex, Inc.

Human Vascular Endothelial Growth Factor Detection Kit

Catalog # 6810

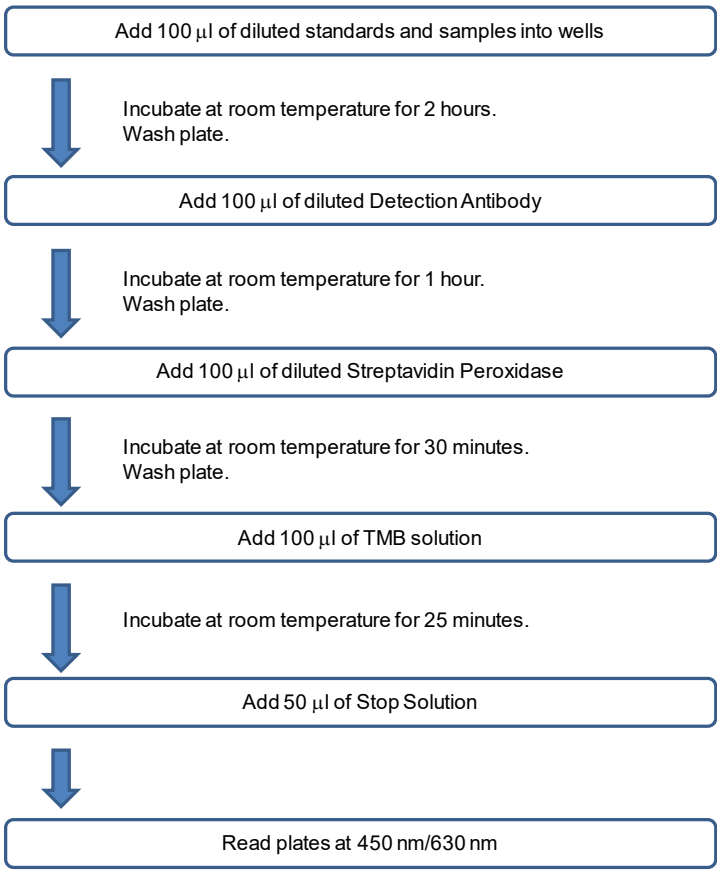
For Research Use Only - Not Human or Therapeutic Use

Chondrex, Inc. provides a human vascular endothelial growth factor (VEGF) quantitative ELISA kit for cell culture media, serum, and plasma samples.

KIT COMPONENTS

Item	Quantity	Amount	Storage
VEGF Standard (68101)	2 vials	2000 pg, lyophilized	-20°C
Detection Antibody (68103)	2 vials	50 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
Capture Antibody Coated 96-Well ELISA Plate	1 each	8-well strips x 12	-20°C

ASSAY OUTLINE



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NOTES BEFORE USING ASSAY

Note 1: It is recommended that the standard and samples be run in duplicate.

Note 2: Warm up all buffers to room temperature before use.

Note 3: Partially used reagents may be kept at –20°C.

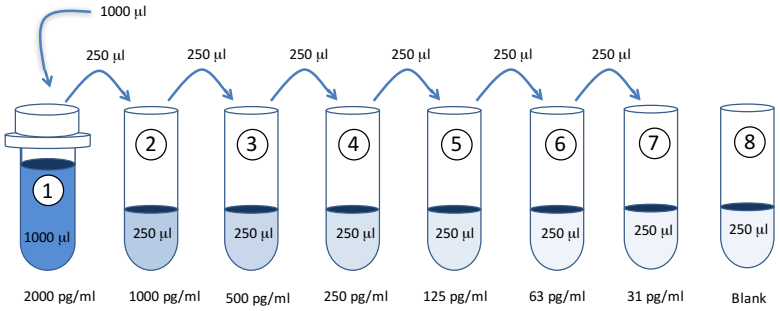
Note 4: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 31-2000 pg/ml. Dissolve one vial of human VEGF standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 2000 pg/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (2000 pg/ml) with an equal volume of Solution B to make a 1000 pg/ml solution, and then repeat it five more times for 500, 250, 125, 63, and 31 pg/ml solutions. The remaining 2000 pg/ml standard stock may be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



2. **Prepare Samples**:

   Cell Culture Media: Remove cell debris and insoluble precipitate by centrifuging at 10,000 rpm for 5 minutes. When not in use, store the supernatant samples at -20°C; avoid repeat freeze-thaw cycles.

   Serum*: Clot blood samples by incubating samples at room temperature for 2 hours. Collect serum by centrifuging samples at 10,000 rpm for 5 minutes. When not in use, store the serum supernatant samples at -20°C; avoid repeat freeze-thaw cycles.

   Plasma*: Collect plasma samples with the use of anticoagulants such as heparin. Collect plasma by centrifuging samples at 10,000 rpm for 5 minutes within 30 minutes of blood collection. When not in use, store the plasma supernatant samples at -20°C; avoid repeat freeze-thaw cycles.

   *Note: Using lipemic (cloudy) samples may affect assay results. The stored samples must be centrifuged at 10,000 rpm for an additional 5 minutes before the assay.

   Dilution: Dilute samples at least 1:1 with Solution B depending on the estimated VEGF level in the samples. Two to three different sample dilutions are recommended if the VEGF levels in the samples are unknown.

   Note: Samples must be diluted with Solution B to maintain optimal assay conditions.

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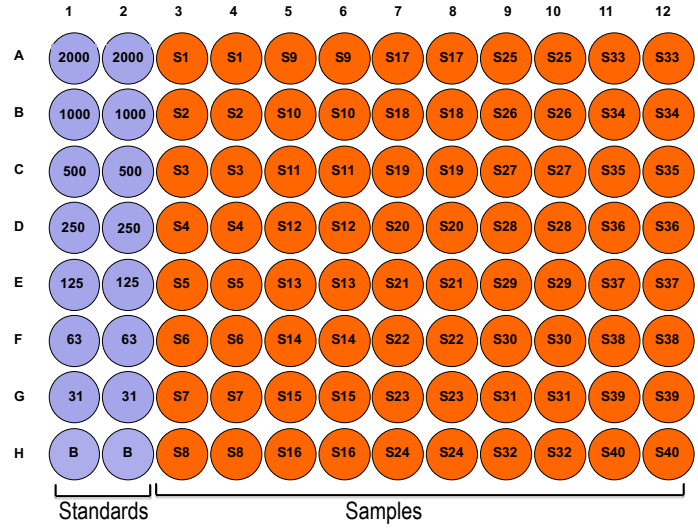
3. **Add Standards and Samples**: Add 100 µl of Solution B (blank), standards, and samples to designated wells in duplicate according to the layout in Figure 1. Incubate at room temperature for 2 hours.

Figure 1 - A Standard Assay Layout



4. **Dilute Wash Buffer**: Dilute 50 ml of Wash Buffer, 20X in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Detection Antibody Solution**: Prepare detection antibody solution with Sample/Standard/Detection Antibody Dilution Buffer (Solution B) as shown in the following table.

Strip #	Detection Antibody (µl)	Solution B (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

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9. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATION OF RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.
2. Subtract the "blank" (B) values from the averaged OD values in step 1.
3. Plot the OD values of standards against the concentration of human VEGF (pg/ml). Using a log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is 32-2000 pg/ml.
4. The pg/ml of VEGF in test samples can be calculated using regression analysis.

Figure 2 - A typical standard curve for human VEGF assay

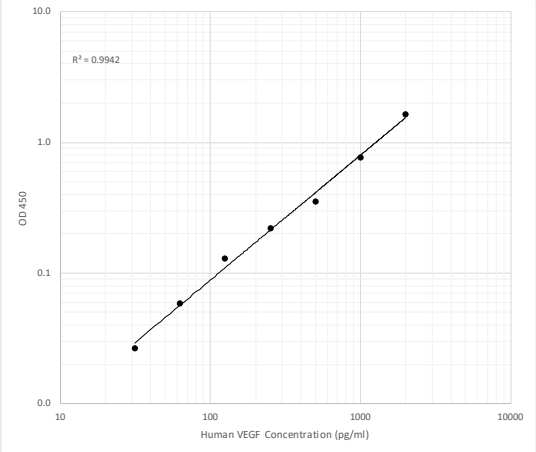

Table 1 - Reproducibility for human VEGF ELISA Kit

Test	125 pg/ml	700 pg/ml
Inter-Assay CV (%)	6.6	10.0
Intra-Assay CV (%)	7.2	8.7
Spike Test*	89%	92%

* Known amounts of human VEGF was added to samples and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

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