Rat VEGF ELISA Kit

NB-06-0955

Rat VEGF ELISA Kit

#Cat: NB-06-0955 Size: 96 tests

For the quantitative measurement of Rat VEGF in cell culture supernatants, serum and plasma (heparin, EDTA, citrate).

Lot to lot variation can occur. Refer to the manual provided with the kit.
This product is intended for research use only.

Principle

Neo Biotech VEGF ELISA Kit (Rat) is based on standard sandwich enzyme-linked immune-sorbent assay technology. An antibody specific for VEGF has been pre-coated onto 96-wellplate (12 x 8 Well Strips). Standards (NSO, A27-R190) and test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for VEGF is added, incubated and followed by washing. Avidin-Biotin-Peroxidase Complex is then added, incubated and unbound complex is washed away. An enzymatic reaction is visualized through the addition of TMB substrate which is catalyzed by HRP to produce a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Rat VEGF captured in the well.

Background

Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF), a potent cytokine expressed by most malignant tumors, has critical roles in vasculogenesis and both physiological and pathological angiogenesis. VEGF produced by tumor cells potently stimulates endothelial cell proliferation and angiogenesis and plays a key role in the pathophysiology of several neoplasias. VEGF may also play a pivotal role in mediating the development and progression of diabetic retinopathy. VEGF, a major regulator of angiogenesis, binds to two receptor tyrosine kinases, KDR/Flk-1 and Flt-1. The VEGF gene is mapped by fluorescence in situ hybridization to chromosome 6p12.The standard product used in this kit is recombinant rat VEGF164, which is a 25KDa single chain as well as a 50kDa dimer.

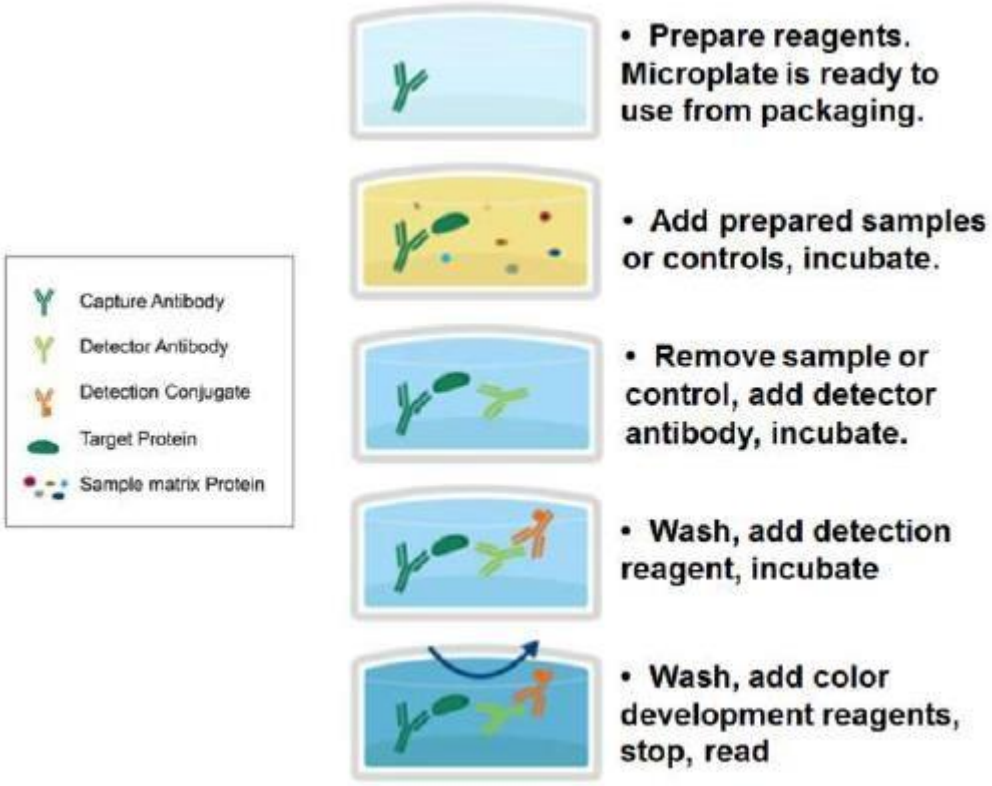
Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For info rmation on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

Storage and Stability

Upon receipt store kit at 4°C for 6 months or -20°C for 12 months. Avoid multiple freeze/thaw cycles

Assay Summary



Materials Provided

Description	Quantity
96-Well plate Pre-coated with Anti-Rat VEGF Antibody	96 Wells (12 x 8 Well Strips)
Lyophilized Recombinant Rat VEGF standard	2 x 10 ng
100X Biotinylated Anti-Rat VEGF Antibody	130 µL
100X Avidin-Biotin-Peroxidase Complex (ABC)	130 µL
Sample Diluent Buffer	30 mL
Antibody Diluent Buffer	12 mL
ABC Diluent Buffer	12 mL
TMB Color Developing Agent	10 mL
TMB Stop Solution	10 mL
10X Wash Buffer	30 mL

Additional Materials Required

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.

Health and Safety Recautions

- Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
- Please carefully review the MSDS for each reagent before conducting the experiment.
- Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Inspect all reagents prior to use. Components should contain no particulates or cloudiness and should be colorless.
- Prior to using the kit, briefly spin component tubes to collect all reagent at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results in inter- intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.
- Pipetting less than 1 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing precipitates or fibrin strands or which are hemolytic of lipemic might cause inaccurate results due to interfering factors.

Reagent Preparation

Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

1X Biotinylated Anti-Rat VEGF Antibody

1. Prepare the 1X Biotinylated Anti-Rat VEGF Antibody immediately prior to use by diluting the 100X Biotinylated Anti-Rat VEGF Antibody 1:100 with Antibody Diluent Buffer.
2. For each well to be used in the experiment prepare 1,000 µL by adding 10 µL of 100X Biotinylated Anti-Rat VEGF Antibody to 990 µL Antibody Diluent Buffer.
3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

1X Avidin-Biotin-Peroxidase Complex (ABC)

1. Prepare the 1X Avidin-Biotin-Peroxidase Complex (ABC) immediately prior to use by diluting the 100X Avidin-Biotin-Peroxidase Complex (ABC) 1:100 with ABC Dilution Buffer.
2. For each well to be used in the experiment prepare 1,000 µL by adding 10 µL of 100X Avidin- Biotin-Peroxidase Complex (ABC) to 99 µL ABC Dilution Buffer.
3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

1X Wash Buffer

1. Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.
2. Add the entire 30 mL contents of the 10X Wash Buffer bottle to the water.
3. Seal and mix gently by inversion. Avoid foaming or bubbles.
4. Store the 1X Wash Buffer at room temperature until ready to use in the procedure. Store the prepared 1X Wash Buffer at 4°C for no longer than 1 week. Do not freeze.

Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

VEGF Assay standards

1. Prepare the VEGF standards no greater than 2 hours prior to performing experiment. standards should be held on ice until use in the experiment.
2. Reconstitute one of the provided 10 ng Lyophilized Recombinant Rat VEGF standard. Use one for each experiment. Prepare a stock 10,000 pg/mL Rat VEGF standard by reconstituting one tube of Lyophilized Recombinant Rat VEGF standard as follows:
   a. Gently spin or tap the vial to collect all material at the bottom.
   b. Add 1 mL of Sample Diluent Buffer to the vial.
   c. Seal then mix gently and thoroughly.
   d. Leave the vial to sit at ambient temperature for 10 minutes.
   3. Prepare a set of seven serially diluted standards as follows:
   a. Label tubes with numbers 1 – 8.
   b. Add 300 µL of Sample Diluent Buffer to Tube #'s 1 – 7.
   c. Prepare a 1,000 pg/mL standard #1 in by adding 100 µL of the 10,000 pg/mL reconstituted Rat VEGF standard to 900 µL of Sample Diluent Buffer in Tube#1. Mix gently and thoroughly.
   d. Prepare standard #2 by adding 300 µL of 1,000 pg/mL standard#1 from Tube #1 to Tube #2. Mix gently and thoroughly.
   e. Prepare standard #3 by adding 300 µL of standard #2 from Tube #2 to Tube #3. Mix gently and thoroughly.
   f. Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.
   g. Tube #8 is a blank standard (only Sample Diluent Buffer), which should be included with every experiment.

Standard Number (Tube)	Sample To Dilute	Volume Standard (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	10,000 pg/mL of Rat VEGF standard	100	900	1,000	1,000 pg/mL
2	Tube#1	300	300	600	500 pg/mL
3	Tube#2	300	300	600	250 pg/mL
4	Tube#3	300	300	600	125 pg/mL
5	Tube#4	300	300	600	62.5 pg/mL
6	Tube#5	300	300	600	31.2 pg/mL
7	Tube#6	300	300	600	15.6 pg/mL
8	NA	0	300	300	0.0 (Blank)

Sample Preparation

Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- Clear samples by centrifugation as follows:
  - Cell culture supernatants - Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
  - Serum - Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
  - Plasma - Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at 1,500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

Sample Dilution

- Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.
- Prepare 150 µL sample for each replicate to be assayed.
- Dilute samples with Sample Diluent Buffer.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Refer to the following table for recommended sample dilution guidelines based on the dynamic range of this kit:

Estimated Sample Target Concentration		Dilution Level	Sample Volume For Two Replicates	Sample Diluent Buffer For Two Replicates
High Concentration	10-100 ng/mL	1:100	1 µL	99 µL
Medium Concentration	1-10 ng/mL	1:10	10 µL	90 µL
Low Concentration	15.6-1000 pg/mL	1:2	50 µL	50 µL
Very Low Concentration	≤ 15.6 pg/mL	1:2 or No Dilution	-	-

Assay Procedure

Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure. Optimal results for intra- and inter-assay reproducibility will be obtained when performing all incubation steps at 37°C as indicated below.

1. Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.
2. Cover the plate with the well plate lid and incubate at 37°C for 90 minutes.
3. Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
4. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
5. Add 100 µL of prepared 1X Biotinylated Anti-Rat VEGF Antibody to each well.
6. Cover with the well-plate lid and incubate at 37°C for 60 minutes.
7. Wash plate 3 times with 1X Wash Buffer as follows:
    a. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
    b. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
    c. Add 300 µL of 1X Wash Buffer to each assay well.
    d. Incubate for 1 minute.
    e. Repeat steps 7a. through 7e. two more times.
8. Add 100 µL of prepared 1X Avidin-Biotin-Peroxidase Complex (ABC) into each well and incubate at 37°C for 30 minutes.
9. Wash plate 5 times with 1X Wash Buffer as follows:
    a. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
    b. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
    c. Add 300 µL of 1X Wash Buffer to each assay well.
    d. Incubate for 1 minute.
    e. Repeat steps 9a. through 9e. four more times.
10. Add 90 µL of TMB Color Developing Agent to each well and incubate at 37°C in the dark for 25- 30 minutes. (NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)
11. Add 100 µL of TMP Stop Solution to each well. Well color should change to yellow immediately.
12. Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 11.

Calculation of Results

For analysis of the assay results, calculate the Relative OD450 for each test or standard well as follows:

(Relative OD_{450}) = (Well OD_{450}) – (Mean Blank Well OD_{450})

The standard curve is generated by plotting the mean replicate Relative OD_{450} of each standard serial dilution point vs. the respective standard concentration. The Rat VEGF concentration contained in the samples can be interpolated by using linear regression of each mean sample Relative OD_{450} against the standard curve. This is best achieved using curve fitting software.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

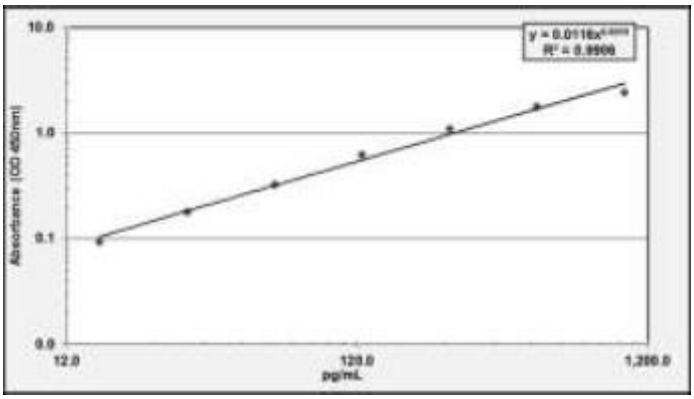
Typical Expected Data

Typical absorbance values. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD_{450}.

Standard Number	8	7	6	5	4	3	2	1
Standard Concentration (pg/mL)	0	15.6	31.2	62.5	125	250	500	1,000
OD_{450}	0.014	0.094	0.179	0.328	0.624	1.098	1.781	2.432

Typical standard curve.

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



General Specifications

Range	15.6 pg/mL -1,000 pg/mL
Sensitivity	< 1 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Natural and recombinant Rat VEGF
UniProt ID:	P16612
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

Reproducibility

	Intra-Assay			Inter-Assay		
Sample ID	1	2	3	1	2	3
n =	16	16	16	24	24	24
Mean Measured Concentration (pg/mL)	120	305	621	236	289	632
Standard Deviation	4.44	15.86	29.81	10.62	18.21	34.76
Consistency (%CV)	3.7	5.2	4.8	4.5	6.3	5.5

References

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