



EAGLE
BIOSCIENCES

Rat FLT4/VEGFR3 ELISA Kit

Catalog Number:

FT421-K01 (1 x 96 wells)

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

v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM



INTENDED USE

The Eagle Biosciences Rat FLT4/VEGFR3 ELISA Kit is intended for the quantitation of Rat FLT4 concentrations in cell culture supernates and serum. The Eagle Biosciences Rat FLT4/VEGFR3 ELISA Kit is for research use only and not for diagnostic or therapeutic procedures.

INTRODUCTION

Fms-related tyrosine kinase 4, also known as FLT4, is a protein which in humans is encoded by the FLT4 gene. It is mapped to 5q35.3. This gene encodes a tyrosine kinase receptor for vascular endothelial growth factors C and D. The protein is thought to be involved in lymphangiogenesis and maintenance of the lymphatic endothelium. FLT4 has an essential role in the development of the embryonic cardiovascular system before the emergence of the lymphatic vessels. It has been found that FLT4, which provides proangiogenic signaling when expressed on endothelium, may also have antiangiogenic properties when expressed at an avascular site by nonendothelial cells. FLT4 is also regarded as a regulator of vascular network formation.

PRINCIPLE OF THE ASSAY

The Rat FLT4 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Rat FLT4 with a 96-well strip plate that is pre-coated with antibody specific for FLT4. The detection antibody is a biotinylated antibody specific for FLT4. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Rat FLT4 with immunogen: Expression system for standard: NSO; Immunogen sequence: Y25-D770. The kit is analytically validated with ready to use reagents. To measure Rat FLT4, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbound ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Rat FLT4 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Rat FLT4 in the sample.

| | |
|-----------------------------|---|
| Reactive Species | Rat |
| Size | 96 wells/kit, with removable strips |
| Description | Sandwich High Sensitivity ELISA kit for Quantitative Detection of Rat FLT4/VEGFR3. 96wells/kit, with removable strips. |
| Sensitivity | <10pg/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 | 156pg/ml-10000pg/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.) |

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 Rat FLT4 |

**Immunogen**

Expression system for standard: NSO; Immunogen sequence: Y25-D770

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 |
|--|----------|----------------------|
| Anti-Rat FLT4 Precoated 96-well strip microplate | 1 | 12 strips of 8 wells |
| Rat FLT4 Standard | 2 | 10ng/tube |
| Rat FLT4 Biotinylated antibody (100x) | 1 | 130 μ l |
| Avidin-Biotin-Peroxidase Complex (100x) | 1 | 130 μ l |
| 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 Powder | 1 | Pack |
| Plate Sealers | 4 | Piece |

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 μ l 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.

STANDARD CURVE EXAMPLE

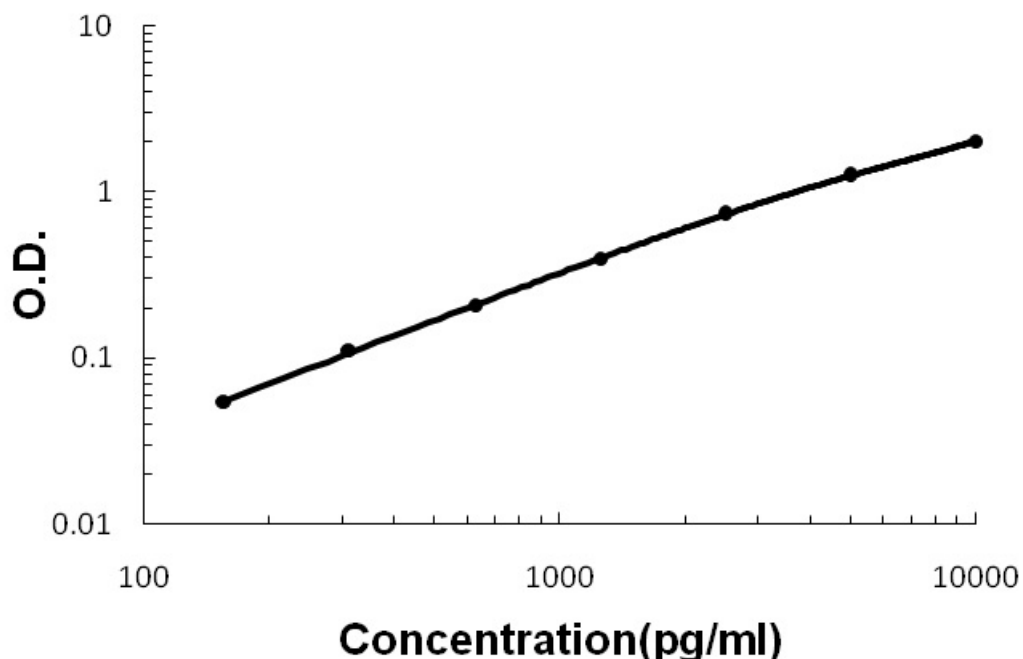
Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

| | | | | | | | | |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Concentration (pg/ml) | 0 | 156 | 312 | 625 | 1250 | 2500 | 5000 | 10000 |
| O.D. | 0.033 | 0.055 | 0.111 | 0.207 | 0.399 | 0.735 | 1.251 | 2.029 |



Rat FLT4/VEGFR3 ELISA Kit Standard Curve

Rat VEGFR3/FLT4 ELISA Kit



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

INTRA/INTER ASSAY VARIABILITY

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.

Intra-Assay Precision

| Sample | 1 | 2 | 3 |
|--------------------|-------|-------|--------|
| N | 16 | 16 | 16 |
| Mean(pg/ml) | 256 | 1651 | 4864 |
| Standard Deviation | 11.77 | 70.99 | 321.02 |
| CV (%) | 4.6% | 4.3% | 6.6% |

Inter-Assay Precision

| Sample | 1 | 2 | 3 |
|--------------------|------|-------|--------|
| N | 24 | 24 | 24 |
| Mean(pg/ml) | 259 | 1599 | 5230 |
| Standard Deviation | 14.5 | 92.74 | 371.33 |
| CV (%) | 5.6% | 5.8% | 7.1% |



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 | 256 | 221 | 252 | 247 | 244 | 13.65 | 5.5% |
| Sample 2 | 1651 | 1702 | 1683 | 1684 | 1680 | 18.37 | 1% |
| Sample 3 | 4864 | 4765 | 4911 | 4762 | 4825 | 64.19 | 1.3% |

*number of samples for each test n=16.

PREPARATION BEFORE THE EXPIREMENT

| Item | Preparation |
|-------------------------------------|---|
| All reagents | Bring all reagents to 37°C prior to use. 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 | Dissolve the wash buffer powder in 1000ml of water to make 1X PBS wash buffer. |
| Biotinylated Anti-Rat FLT4 antibody | It is recommended to prepare this reagent immediately prior to use by diluting the Rat FLT4 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl 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. |
| Rat FLT4 Standard | It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Rat FLT4 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/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 |



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| | unused well strips should be sealed and stored in the original packaging. |
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DILUTION OF RAT FLT4 STANDARD

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –10000pg/ml, #2 –5000pg/ml, #3 - 2500pg/ml, #4 – 1250pg/ml, #5 – 625pg/ml, #6 –312.5pg/ml, #7 – 156.25pg/ml, #8 – 0.0 (Blank).
2. For standard #1, add 1000µl of undiluted standard stock solution to tube #1.
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. |

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.

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 is 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 μ l of the prepared 1x Biotinylated Anti-Rat FLT4 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 μ l 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 μ l 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 μ l 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 μ l 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 μ l 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-logisticcurve.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.



Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.