Package Insert

Mouse Flt-3ligand ELISA Kit

Catalog Number: FT311-K01

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

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INTENDED USE

The Eagle Biosciences Mouse FLT-3ligand ELISA Kit is intended for the quantitation of Mouse FLT3LG concentrations in cell culture supernates and serum. The Eagle Biosciences Mouse FLT3ligand ELISA Kit is for research use only and not for diagnostic or therapeutic procedures. *For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.*

INTRODUCTION

Fms-related tyrosine kinase 3 ligand (FLT3LG), also known as Flt-3ligand or FL, is a protein which in humans is encoded by the FLT3LG gene. It is mapped to 19q13.3. Flt-3ligand is a hematopoietic four helical bundle cytokine. It is structurally homologous to stem cell factor (SCF) and colony stimulating factor 1(CSF-1). In synergy with other growth factors, Flt-3ligand stimulates the proliferation and differentiation of various blood cell progenitors. Besides that, Flt-3ligand can control the development of Dendritic cells (DCs) and is particularly important for plasmacytoid DCs and CD8-positive classical DCs and their CD103-positive tissue counterparts. Flt-3ligand also can enhance the response of stem and primitive progenitor cells to other growth factors to generate all myeloid lineages except erythroid cells.

ASSAY PRINCIPLE

The Mouse FLT3LG Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse FLT3LG with a 96-well strip plate that is precoated with antibody specific for FLT3LG. The detection antibody is a biotinylated antibody specific for FLT3LG. The capture antibody is monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Mouse FLT3LG with immunogen: Expression system for standard: NSO; Immunogen sequence: G27-R188. The kit is analytically validated with ready to use reagents.

To measure Mouse FLT3LG, 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 unbounded 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 Mouse FLT3LG 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 Mouse FLT3LG in the sample.

OVERVIEW

Reactive Species	Mouse
Size	96/wells / Kit with removable strips
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Mouse FLT-3ligand. 96wells/kit, with removable strips.
Sensitivity	< 10 pg/ml
Detection Range	31.2 pg/ml – 2000 pg/ml

Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.}

TECHNICAL DETAILS

Capture/Detection Antibodies	The capture antibody is monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat.
Specificity	Natural and recombinant Mouse FLT3LG
Immunogen	Expression system for standard: NSO; Immunogen
Cross-reactivity	There is no detectable cross-reactivity with other relevant proteins.

ASSAY PREPARATION

Please read the following instructions before starting the experiment.

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the kit, spin tubes to bring down all components to the bottom of the tubes.
9. Don't let the 96-well plate dry out since this will inactivate active components on the plate.
10. Don't reuse tips and tubes to avoid cross-contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

MATERIALS PROVIDED

Description	Quantity	Volume	Storage of opened / Reconstituted material
Anti-Human FLT3G 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 FLT3G Standard	2	10 ng/tube	Discard the FLT3G stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.
Human FLT3G Biotinylated Antibody (100X)	1	100µl	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	100µl	
Sample Diluent	1	30mL	
Antibody Diluent	1	12 ml	
Avidin-Biotin-Peroxidase Diluent	1	12 ml	
Color Developing Reagent (TMB)	1	10 ml	
Stop Solution	1	10 ml	
Wash Buffer (25X)	1	20 ml	

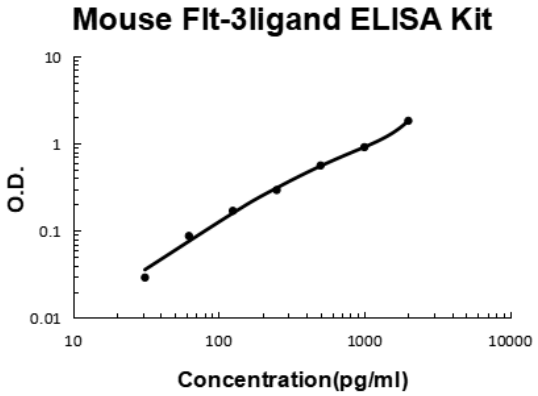
MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading absorbance at 450 nm.
- Incubator.
- 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 a large numbers of samples.
- Deionized or distilled water.
- 500 ml graduated cylinders.
- Test tubes for dilution

TYPICAL CALIBRATOR CURVE

The 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	31.2	62.5	125	250	500	1000	2000
O.D.	0.030	0.059	0.117	0.199	0.323	0.590	0.939	1.854



PERFORMANCE AND CHARACTERISTICS

Intra-Assay

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay

Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Intra-Assay			
Sample	1	2	3
N	16	16	16
Mean (pg/ml)	66	243	884
Standard Deviation	3.3	10.44	59.22
CV %	5%	4.3%	6.7%

Inter-Assay			
Sample	1	2	3
N	24	24	24
Mean (pg/ml)	69	243	901
Standard Deviation	4.27	14.58	64.87
CV %	6.2%	6%	7.2%

REPROCUIBILITY

We ensure reproducibility by testing three samples with differing concentrations of F11 in ELISA kits from four different production batches/lots.

Lots	Lot 1 (pg/ml)	Lot 2 (pg/ml)	Lot 3 (pg/ml)	Lot 4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	66	73	74	70	70	3.11	4.4%
Sample 2	243	247	257	224	242	11.96	4.9%
Sample 3	884	811	863	831	847	28.18	3.3

Number of samples for each test n=16

REAGENT PREPARATION

Component	Preparation
All Reagents	Bring all reagents to room temperature (18-25°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 (15-25 min) is based on incubation at 37°C.
Wash Buffer	Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml 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-Mouse FLT3LG antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Mouse FLT3LG Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µI by adding 1 µI of Biotinylated antibody (100x) to 99 µI 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-BiotinPeroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation..
Mouse FLT3LG Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Mouse FLT3LG 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.
Samples	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Internal QC testing used: Dilution ratio of 1: 5, concentration in serum and plasma is around 1000 ng/ml.

DILUTION OF HUMAN CD274 STANDARD

1. Number tubes 1-8. Final Concentrations to be
    a. # 1: 2,000.00 pg/ml
    b. # 2: 1,000.00 pg/ml
    c. # 3: 500.00 pg/ml
    d. # 4: 2500.00 pg/ml
    e. # 5: 125.00 pg/ml
    f. # 6: 62.50 pg/ml
    g. # 7: 31.25 pg/ml
    h. # 8: Sample Diluent serves as the zero standard (0 pg/ml).

2. For standard #1, add 200 µl of the reconstituted standard stock solution of 10 ng/ml and 800µl of sample diluent to tube #1 for a final volume of 1000µl. 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.

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 dilution ratios should be determined by a pilot study (run a serial dilution of samples and see which dilution ratio results in the idea O.D., near the middle of the standard range). In general, high concentration samples can be diluted by 1:100, mid concentration samples 1:10, low concentration samples 1:2 or neat**.**

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 COLLECTION NOTES

1. Eagle Biosciences recommends that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation

   experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

8. Eagle Biosciences is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

SAMPLE DILUTION GUIDELINE

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type are necessary. The sample must be mixed thoroughly with Sample Diluent.

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 µl** of the prepared **1x Biotinylated Anti-Mouse FLT3LG 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.

ASSAY PROTOCOL NOTES

1. Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
2. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
3. Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
4. Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
5. Washing: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
6. Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. The excessively strong color will result in inaccurate absorbance readings.
7. Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking O.D. readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
8. Reaction Time Control: Control reaction time should be strictly followed as outlined.

9. Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.
10. To minimize the external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

REFERENCES

1. PubMed ID: 25674205, Chen Yf, Zhao Zq, Wu Zm, Zou Zy, Luo Xj, Li J, Xie C, Liang Y. Int J Clin Exp Pathol. 2014 Dec 1;7(12):8411-20. Ecollection 2014. The Role Of Rip1 And Rip3 In The Development Of Aplastic Anemia Induced By Cyclophosphamide And Busulphan In Mice.

CALCULATION OF RESULTS

To analyze using manual methods, follow the process below:
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 O.D. 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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