

Quantikine[®] ELISA

Mouse/Rat Flt-3 Ligand Immunoassay

Catalog Number MFK00

For the quantitative determination of mouse or rat Flt-3 Ligand concentrations in cell culture supernates and serum.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Flt-3 Ligand is a ligand for the receptor tyrosine kinase Flt-3/Flk-2 (*fms*-like tyrosine kinase/fetal liver kinase) and is structurally related to macrophage colony-stimulating factor (M-CSF) and stem cell factor (SCF) (1-3). Flt-3 Ligand has been shown to synergize with a wide variety of hematopoietic cytokines to stimulate the growth and differentiation of early hematopoietic progenitors (4). Flt-3 Ligand also stimulates dendritic cell development (4-7).

The gene for mouse Flt-3 Ligand is composed of 8 exons and can give rise to multiple isoforms (3-8). The largest active isoform is a 231 amino acid (aa) residue transmembrane protein that is biologically active on the cell surface (2-3). Under certain conditions, this 231 aa membrane protein can be proteolytically cleaved to generate a soluble form of biologically active Flt-3 Ligand (3, 9). A 220 aa membrane-anchored mouse Flt-3 Ligand exists. This 220 aa isoform lacks the proteolytic cleavage site present in the 231 aa isoform but is biologically active on the cell surface (10, 11). An alternatively spliced mRNA encoding a soluble form of biologically active mouse Flt-3 Ligand containing 169 aa residues has been identified (3, 10). Another 252 aa residues transmembrane mouse Flt-3 Ligand isoform lacking the last of four conserved cysteine residue has also been reported. This isoform is found to be biologically inactive (10). Flt-3 Ligand is expressed widely in many tissues (9). In bone marrow and spleen, the most prevalent isoform is the biologically active 231 aa transmembrane protein (8). Among primary cell types, those reported to express Flt-3 Ligand include myeloid, monocytic, megakaryocytic, erythroid and B-cell lines (11), colorectal tumor cells (13), fibroblasts (14), endothelial cell (15) and keratinocytes (16). At the amino acid sequence level, human and mouse Flt-3 Ligand are approximately 72% identical and the two proteins exhibit cross-species activity (1, 12).

The receptor for Flt-3 Ligand, Flt-3/Flk-2, belongs to the same subfamily of receptor tyrosine kinases as *c-fms* (M-CSF receptor), *c-kit* (SCF receptor) and the two PDGF receptors (17, 18). Each of these receptors is a type I transmembrane glycoprotein that has five immunoglobulin-like domains in its extracellular region and a split kinase domain in its cytoplasmic region (19). The expression of mouse Flt-3/Flk-2 is limited to brain, bone marrow and a small subpopulation of early hematopoietic progenitor cells (19).

The Quantikine® Mouse/Rat Flt-3 Ligand Immunoassay is a 4.5 hour solid phase ELISA designed to measure Flt-3 Ligand levels in mouse or rat cell culture supernates and serum. It contains NS0-expressed recombinant mouse Flt-3 Ligand and antibodies raised against the recombinant protein. Results obtained for naturally occurring mouse or rat Flt-3 Ligand showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values of mouse/rat Flt-3 Ligand.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse/rat Flt-3 Ligand has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Flt-3 Ligand present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat Flt-3 Ligand is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of Flt-3 Ligand bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- 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.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat Flt-3 Ligand Microplate	890822	96 well microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse/rat Flt-3 Ligand.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat Flt-3 Ligand Standard	890824	Recombinant mouse Flt-3 Ligand in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.*
Mouse/Rat Flt-3 Ligand Control	890391	Recombinant mouse Flt-3 Ligand in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse/Rat Flt-3 Ligand Conjugate	890823	12 mL of a polyclonal antibody specific for mouse/rat Flt-3 Ligand conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	12 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-17	895512	21 mL of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of a buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed or lipemic samples are not suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse/Rat Flt-3 Ligand Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

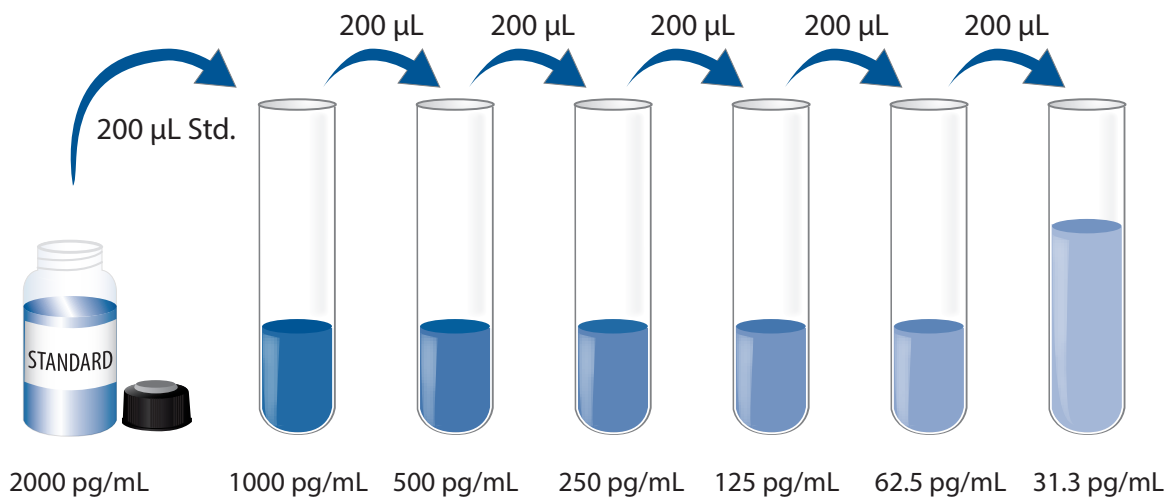
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 μ L of the resultant mixture is required per well.

Mouse/Rat Flt-3 Ligand Standard - Refer to the vial label for reconstitution volume.

Reconstitute the Mouse/Rat Flt-3 Ligand Standard with Calibrator Diluent RD5-17. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 μ L of Calibrator Diluent RD5-17 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse/Rat Flt-3 Ligand Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5-17 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare reagents and standard dilutions as directed by the previous section.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 μL of Assay Diluent RD1W to each well.
4. Add 50 μL of standard, control, or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 μL of Mouse/Rat Flt-3 Ligand Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

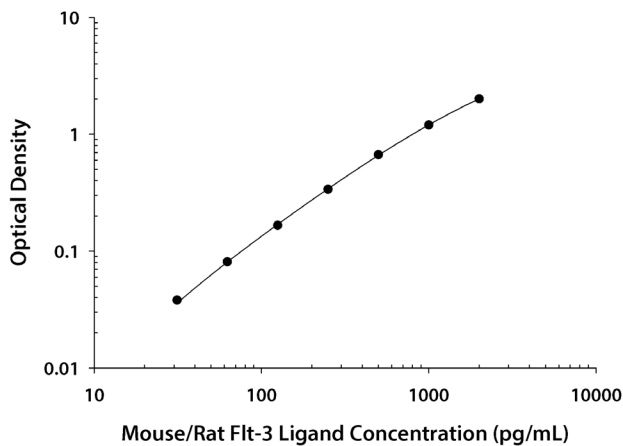
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse/rat Flt-3 Ligand concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.028 0.029	0.028	—
31.3	0.066 0.065	0.066	0.038
62.5	0.110 0.108	0.109	0.081
125	0.193 0.196	0.194	0.166
250	0.357 0.376	0.366	0.338
500	0.696 0.694	0.695	0.667
1000	1.194 1.258	1.226	1.198
2000	1.974 2.094	2.034	2.006

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	79.5	237	775	82.4	242	768
Standard deviation	3.9	10.1	28.6	6.2	13.6	39.4
CV (%)	4.9	4.3	3.7	7.5	5.6	5.1

RECOVERY

The recovery of mouse/rat Flt-3 Ligand spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture supernates (n=5)	99	88-107%
Serum (n=5)	95	82-106%

Rat Samples	Average % Recovery	Range
Cell culture supernates (n=5)	108	99-119%
Serum (n=5)	95	86-114%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse/rat Flt-3 Ligand in each matrix were diluted with calibrator diluent and assayed.

		Mouse Samples		Rat Samples	
		Cell culture supernates (n=5)	Serum (n=5)	Cell culture supernates (n=5)	Serum (n=5)
1:2	Average % of Expected	92	98	92	101
	Range (%)	88-97	95-104	89-95	96-107
1:4	Average % of Expected	95	98	92	103
	Range (%)	90-101	93-106	89-95	97-109
1:8	Average % of Expected	95	102	92	104
	Range (%)	90-100	95-111	87-97	98-110
1:16	Average % of Expected	99	108	90	103
	Range (%)	96-102	99-119	84-97	97-110

SENSITIVITY

The minimum detectable dose (MDD) of mouse/rat Flt-3 Ligand is typically less than 5 pg/mL. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse Flt-3 Ligand produced at R&D Systems®.

SAMPLE VALUES

Serum - Samples were evaluated for the presence of mouse/rat Flt-3 Ligand in this assay.

	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum (n=40)	388	245-533	72
Rat serum (n=5)	393	371-421	22

Cell Culture Supernates - Mouse splenocyte conditioned media (1 x 10⁶ cells/mL) was cultured for 3 days in RPMI supplemented with 10% fetal bovine serum. The cell culture supernate was removed, assayed for mouse Flt-3 Ligand, and measured 222 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse Flt-3 Ligand. This assay also recognizes natural rat Flt-3 Ligand.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse/rat Flt-3 Ligand control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10	IL-9
Eotaxin	IL-10
G-CSF	IL-10 R
GM-CSF	IL-12 p40
IFN-γ	IL-12 p70
IL-1α	IL-13
IL-1β	IL-17
IL-1ra	IL-18
IL-2	JE/MCP-1
IL-3	KC
IL-4	Leptin
IL-5	LIF
IL-6	MARC
IL-7	M-CSF

Recombinant human:

Flt-1
Flt-3 Ligand
G-CSF
PIGF
VEGF

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