Quantikine® ELISA

Human LIF Immunoassay

Catalog Number DLF00

For the quantitative determination of human Leukemia Inhibitory Factor (LIF) concentrations in cell culture supernates, serum, and plasma.

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

Leukemia Inhibitory Factor (or LIF) is a variably glycosylated, 38-67 kDa polypeptide originally identified as a proliferation inhibitor and differentiation inducer of the mouse M1 myeloid leukemia cell line (1-3). The mature LIF molecule measures 180 amino acid (aa) residues in length, with multiple potential N-linked and O-linked glycosylation sites plus six conserved cysteines that form three intramolecular disulfide bridges (1, 4, 5). Mature mouse LIF is 78% identical to human LIF at the aa sequence level (4, 5). Although such homology might suggest a high conservation of LIF biology, notable differences exist between the reported forms of mouse and human LIF proteins and their receptors. For example, alternative splice events are known to occur in mice, but not humans, creating two isoforms of secreted LIF (2). Also, three isoforms of the LIF receptor α-chain, two soluble forms and one transmembrane form, have been reported in mice (6, 7), but not in humans. Based on its helical structure, LIF is considered to be a member of the Interleukin-6 family of cytokines (8). Cells known to express LIF include activated T-cells, monocytes, and astrocytes (2), osteoblasts (9), keratinocytes (10), regenerating skeletal muscle (11), mast cells (12), and fibroblasts (2, 13).

The receptor for LIF (LIF R) belongs to the hematopoietin superfamily of receptors (14). Members of this family (at a minimum) exhibit highly conserved cysteine residues plus a WS-X-WS aa sequence motif within a short 200 aa residue stretch (14). In humans, the LIF R is a 190 kDa, 1053 aa residue type I (external N-terminus) transmembrane glycoprotein. Its extracellular region shows both hematopoietin receptor domains and fibronectin type III modules and its cytoplasmic domain is rich in serine, threonine, and proline (15). When expressed alone, LIF R binds LIF with low affinity (Kd = 1-3 nM) (15, 16). When expressed with gp130, the 130 kDa "affinity-converting" signal-transducing subunit common to the receptor complexes for members of the IL-6 family, a high-affinity tripartite complex forms between LIF R, LIF, and gp130 (Kd = 10-200 pM) (15-18). Utilization of the LIF R is not restricted to LIF. OSM (via its type I receptor) (19), cardiotrophin-1 (20) and CNTF (21) all utilize the LIF R in one form or another. Additional yet undiscovered LIF R ligands are also hypothesized to exist (22). Cells known to express the LIF R at various levels include hepatocytes (16, 22), keratinocytes (10), monocytes, osteoblasts, melanoma cell lines (3, 23, 24), and macrophages (16, 23).

Functionally, LIF has been implicated in a number of processes including development, hematopoiesis, bone metabolism, and inflammation (3). Given the diversity of ligands associated with the LIF R, it is presently unclear which effects are strictly attributable to LIF versus those which may be due to general LIF R binding, or those mediated by other cytokine receptor superfamily members (22, 25-27). LIF has been detected in a variety of body fluids. Elevated concentrations (pg/mL) of LIF in serum have been correlated with the presence of hematologic malignancy (lymphoma) (28), while elevated levels in bronchoalveolar lavage have been correlated with an increase in markers of inflammation (29). Elevated concentrations (ng/mL) of LIF have been correlated with the peripheral white cell count in rheumatoid arthritis patients (30).

The Quantikine Human LIF Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure soluble human LIF in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human LIF and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant LIF accurately. Results obtained measuring natural human LIF showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human LIF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human LIF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any LIF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme linked polyclonal antibody specific for human LIF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of LIF bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
Human LIF Microplate	890048	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human LIF.	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.*
Human LIF Standard	890050	10 ng of recombinant human LIF in a buffered protein base with preservatives; lyophilized.	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human LIF Conjugate	890049	21 mL of a polyclonal antibody specific for human LIF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1U	895138	6 mL of a buffered protein base with preservatives. *May contain a precipitate. For serum/plasma samples.*	
Calibrator Diluent RD5-5	895485	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6L	895011	21 mL of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric 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.
- Human LIF Controls (optional; available from R&D Systems).

PRECAUTIONS

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

Some components in this kit contain ProClin® 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. Please 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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

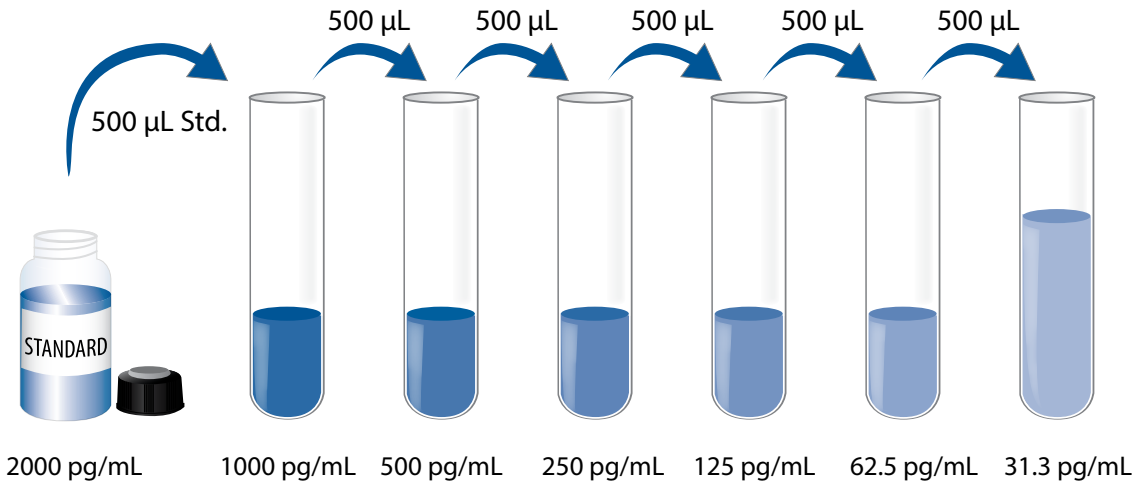
Bring all reagents to room temperature before use.

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. 200 µL of the resultant mixture is required per well.

Human LIF Standard - Reconstitute the Human LIF Standard with 5.0 mL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6L (*for serum/plasma samples*). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of the appropriate Calibrator Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (2000 pg/mL). The appropriate Calibrator Diluent 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, samples, and controls be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. **For Serum/Plasma Samples Only:** Add 50 µL of Assay Diluent RD1U to each well. *Assay Diluent RD1U may contain a precipitate. Mix well before and during its use.*
4. Add 200 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. **For Cell Culture Supernate Samples:** Incubate for 2 hours at room temperature. **For Serum/Plasma Samples:** Incubate for 2 hours at 37 °C.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 200 µL of Human LIF Conjugate to each well. Cover with the adhesive strip provided. **For Cell Culture Supernate Samples:** Incubate for 1 hour at room temperature. **For Serum/Plasma Samples:** Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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.).

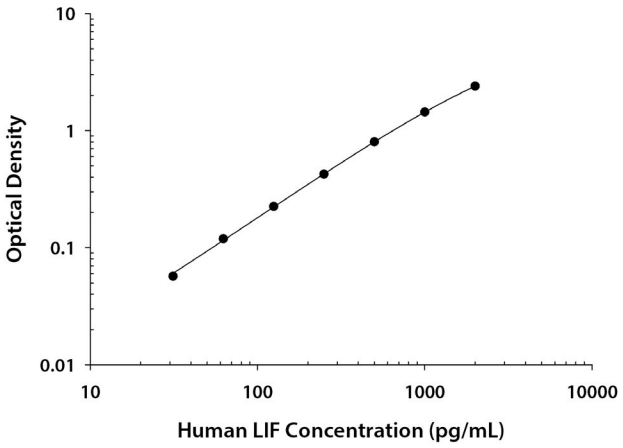
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human LIF 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

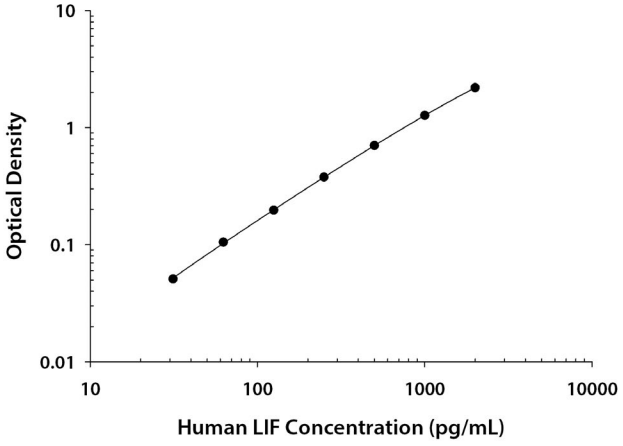
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.029	0.028	—
31.3	0.084 0.086	0.085	0.057
62.5	0.148 0.146	0.147	0.119
125	0.254 0.252	0.253	0.225
250	0.450 0.456	0.453	0.425
500	0.823 0.833	0.828	0.800
1000	1.466 1.466	1.466	1.438
2000	2.483 2.359	2.421	2.393

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.031 0.028	0.030	—
31.3	0.083 0.079	0.081	0.051
62.5	0.135 0.135	0.135	0.105
125	0.232 0.222	0.227	0.197
250	0.417 0.397	0.407	0.377
500	0.723 0.741	0.732	0.702
1000	1.297 1.298	1.298	1.268
2000	2.258 2.180	2.219	2.189

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 components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	341	875	1358	81.6	472	910
Standard deviation	12.3	34.5	42.5	4.4	17.7	35.2
CV (%)	3.6	3.9	3.1	5.4	3.8	3.9

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	314	868	1347	87.4	524	1017
Standard deviation	7.7	24.3	23.8	6.2	34.9	46.5
CV (%)	2.5	2.8	1.8	7.1	6.7	4.6

RECOVERY

The recovery of human LIF spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media	97	91-106%
Serum	102	91-110%
EDTA plasma	89	84-97%
Heparin plasma	92	84-100%

SENSITIVITY

The minimum detectable dose (MDD) of human LIF is typically less than 8 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human LIF. The NIBSC/WHO Reference Reagent 93/562 was evaluated in this kit.

The dose response curve of the NIBSC standard 93/562 parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine Human LIF kit to approximate NIBSC 93/562 Units, use the equation below.

NIBSC/WHO (93/562) approximate value (U/mL) = 0.0229 x Quantikine Human LIF value (pg/mL)

SAMPLE VALUES

Serum - Forty serum samples from apparently healthy volunteers were evaluated for the presence of human LIF in this assay. Thirty-nine samples measured less than the lowest Human LIF standard, 31.3 pg/mL, and one sample measured 44.7 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated for 5 days with 10 µg/mL PHA. An aliquot of the cell culture supernate was removed, assayed for levels of natural human LIF, and measured 1000 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human LIF.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhLIF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

G-CSF
GM-CSF
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-6
IL-7
IL-8
OSM
TGF-β1
TNF-α
TNF-β

Recombinant mouse:

IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7
GM-CSF

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1.2
porcine TGF-β2

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