

HUMAN LEKUMIA INHIBITORY FACTOR (LIF) LIF ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN LIF CONCENTRATIONS IN SERUM AND
EDTA PLASMA



FOR RESEARCH USE ONLY. NOT FOR USE IN
DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN LIF ELISA
Catalog No.	SK00355-01
Lot No.	
Formulation	96 T
Standard range	3.125-400 ng/ml
Sensitivity	200 pg/ml
Sample require	100 µl
Dilution Factor	<i>Optimal dilutions should be determined by each laboratory for each application)</i>
Sample Type	Serum, EDTA Plasma
Specificity	Human LIF
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	4 °C

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INTRODUCTION

Human Lekemia Inhibitory Factor (LIF) Immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human LIF in serum and plasma. It contains recombinant Human LIF and antibodies raised against this protein. It has been shown to accurately quantify recombinant Human LIF. Results obtained with naturally occurring LIF samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the Immunoassay 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. An monoclonal antibody specific for soluble human LIF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human LIF present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody specific for soluble human LIF is added to the wells. Following a wash to remove any unbound antibody reagent, a Anti Rabbit IgG HRP conjugate is added to the wells. After washing away any unbound enzyme, 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

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_ 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 Dilution Buffer 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 Dilution Buffer 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.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors

have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
LIF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal against human LIF.	355-01-01	1 plate
LIF Standard – 400 ng/vial of recombinant Human LIF in a buffered protein base with preservatives; lyophilized.	355-01-02	1 vial
Detection Antibody – 1.2 mL / vial, 10-fold concentrated of a purified antibody against human LIF with preservatives; lyophilized.	355-01-03	1 vial
Positive Control – one vial of recombinant soluble human LIF , lyophilized	355-01-04	1 vial
Anti Rabbit IgG-HRP Conjugate -60 µl/vial, 200-fold concentrated solution of Streptavidin-HRP Conjugate	SAHRP	1 vial
Dilution Buffer - 60mL of buffered protein based solution with preservatives	DB08	1 bottle
Wash Buffer -50 ml of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution -11 ml of TMB substrate solution	TMB01	1 bottle
Stop Solution - 11 ml of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrated should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Antibody Solution Concentrate SHOULD BE STORED at -20°C or -70°C for up to one

month. SAHRP Conjugate 200-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch (P01) with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted Hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

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, heparin, or citrate 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.

Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.

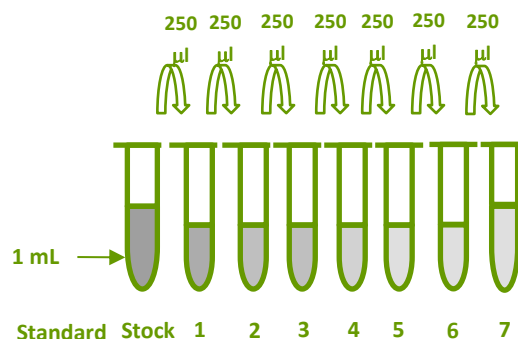
REAGENT PREPARATION

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. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

LIF Standard - Refer to vial label for reconstitution volume. Reconstitute the LIF Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 400 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 400 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1 ml	400 ng/ml
# 1	250µl of stock	250µl	200 ng/ml
# 2	250µl of 1	250µl	100 ng/ml
# 3	250µl of 2	250µl	50 ng/ml
# 4	250µl of 3	250µl	25 ng/ml
# 5	250µl of 4	250µl	12.5 ng/ml
# 6	250µl of 5	250µl	6.25 ng/ml
# 7	250µl of 6	250µl	3.125 ng/ml



Concentration 400 200 100 50 25 12.5 6.25 3.12 ng/ml

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of the appropriate Dilution Buffer into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Anti Rabbit IgG-HRP Conjugate - Transfer 60 µL of 200-fold concentrated Anti Rabbit IgG-HRP conjugate stock solution to 11.94 mL of **Dilution Buffer** to prepare working solution. *Note: 1 x working solution of Streptavidin HRP Conjugate should be used within a few days.*

Positive Control- Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. *Positive Control should be prepared and used immediately.* Reconstituted Positive Control CAN NOT BE REUSED.

ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (A2, A3).
4. Add 100 µL of Standard solution from #7 to S (reverse order of serial dilution) (from B2, B3 to G2, G3 and G4, G5 to F4, F5), sample, or positive control per well (E4, E5). Cover with the Sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 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 Detection Antibody working solution to each well. Cover with sealer. Incubate

for 2 hours on micro-plate shaker at room temperature.

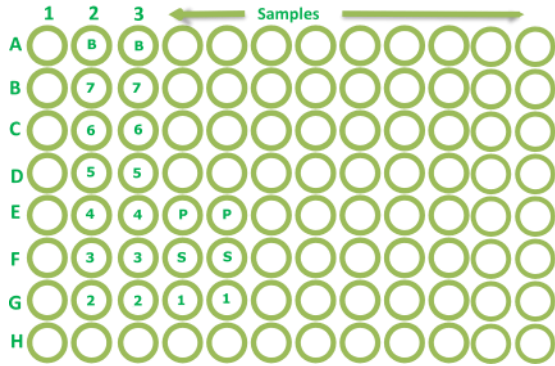
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Anti Rabbit IgG-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-6 minute at room temperature. **Protect from light.**
11. Add 100 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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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 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.

Calculation of samples with a concentration exceeding that of standard 400 ng/ml may result in inaccurate, low human LIF levels. Such samples require further external predilution according to expected human LIF values with Dilution Buffer in order to precisely quantitate the actual human LIF level.



CALIBRATION

This immunoassay is calibrated against a highly purified recombinant Human LIF.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of LIF Was 200 pg/mL.

SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human LIF	100%
Human MIA	0
Human ECP	0
Human Fetuin A	0

TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.160)
3.12	0.026
6.25	0.065
12.5	0.128
25	0.235
50	0.431
100	0.897
200	1.452
400	2.453

LINEARITY

To assess the linearity of the assay, pooled human serum samples were diluted with Dilution Buffer DB08 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1 x	17.926	17.926	100
2 x	8.104	16.208	90.4

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

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Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Anti Rabbit IgG HRP conjugate to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

↓

Add 100 µl Substrate Solution to each well. Incubate 3-6 min plate shaker. **Protect from light.**

↓

Add 100 µl Stop Solution to each well. Read 450nm within 15 min