ORIGENE

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Mouse LIF ELISA Kit

Catalog Number: EA102196

Assay Principle

The OriGene Mouse Lif Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Lif with a 96-well strip plate that is pre-coated with antibody specific for Lif. The detection antibody is a biotinylated antibody specific for Lif. The capture antibody is a monoclonal antibody from rat, the detection antibody is a biotinylated polyclonal antibody from goat. The kit contains recombinant Mouse Lif with immunogen: Expression system for standard: E.coli; Immunogen sequence: S24-F203. The kit is analytically validated with ready to use reagents.

To measure Mouse Lif, 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 Lif 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 Lif in the sample.

Overview

Product Name	Mouse LIF ELISA Kit
Reactive Species	Mouse
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Mouse LIF. 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	7.8pg/ml-500pg/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.)
Uniprot ID	P09056

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Technical Details

Capture/Detection Antibodies	*The capture antibody is a monoclonal antibody from rat, the detection antibody is a biotinylated polyclonal antibody from goat.*
Specificity	*Natural and recombinant Mouse Lif*
Immunogen	*Expression system for standard: E.coli; Immunogen sequence: S24-F203*
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-Mouse Lif Pre-coated 96-well strip microplate	1	12 strips of 8 wells
Mouse Lif Standard	2	1ng/tube
Mouse Lif Biotinylated antibody (100x)	1	130 µl
Avidin-Biotin-Peroxidase Complex (100x)	1	130 µl
Sample Diluent	1	30ml
Antibody Diluent	1	12ml
Avidin-Biotin-Peroxidase Diluent	1	12ml
Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Plate Sealers	4	Piece

**Why there is no wash buffer? Our Avidin-Biotin-Peroxidase Diluent contains the detergent (TWEEN) normally present in other companies' ELISA kits. This saves you the step of having to wash with the special wash buffer and achieve similar or better signal to noise ratio. The wash can use regular wash buffers (PBS, TBS etc.) commonly found in labs.*

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Required Materials That Are Not Supplied

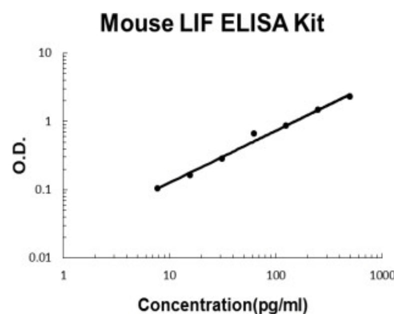
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
1000ml of 1X wash buffer (TBS or PBS)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Mouse LIF ELISA Kit (EA102196) 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**	**7.8**	**15.6**	**31.3**	**62.5**	**125**	**250**	**500**
O.D.	**0.004**	**0.104**	**0.163**	**0.285**	**0.654**	**0.857**	**1.472**	**2.306**

Mouse LIF ELISA Kit standard curve



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

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

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*

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precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	16	70	272	15	76	278
Standard deviation	0.7	3.01	19.58	0.72	3.34	24.74
CV(%)	4.4%	4.3%	7.2%	4.8%	4.4%	8.9%

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	16	15	16	15	15	0.5	3.3%
Sample 2	70	65	74	67	69	3.39	4.9%
Sample 3	272	305	287	293	289	11.88	4.1%

*number of samples for each test n=16.

Preparation Before The Experiment

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	Prepare 1000ml of 1X PBS or TBS for wash buffer.
Biotinylated Anti-Mouse Lif antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Mouse Lif Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100µl by adding 1µl of Biotinylated antibody (100x) to 99µl 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 100µl by adding 1µl of Avidin-Biotin-Peroxidase Complex (100x) to 99µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Mouse Lif Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the

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	experiment. Use one 1ng of lyophilized Mouse Lif standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 1ng/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 unused well strips should be sealed and stored in the original packaging.

Dilution of Mouse Lif Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –500pg/ml, #2 –250pg/ml, #3 – 125pg/ml, #4 – 62.5pg/ml, #5 – 31.25pg/ml, #6 – 15.625pg/ml, #7 – 7.8125pg/ml, #8 – 0.0 (Blank).
2. To generate standard #1, add 500μl of the reconstituted standard stock solution of 1ng/ml and 500μl 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.
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.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

*Note: To detect Lif in samples, you need to activate Lif in samples prior to the assay.

LIF is mostly contained as inactive form in samples, please activate it before assay. Don't activate recombinant LIF.
Solution A: 1N HCl: add 8.33ml of 12N HCl into 91.67ml of H_2O.
Solution B: 1.2N NaOH/0.5M HEPES: add 12ml of 10N NaOH and 11.9g HEPES into 75ml of H_2O, add H_2O to adjust volume to 100ml.

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Activate the sample

Serum, plasma(EDTA): add activating reagent pro rata, i.e. add 20μl of Solution A into 40μl of sample, 10 min later, add 20μl of Solution B. PH 7.0-7.6.

It is unnecessary to activate the Cell culture supernate.

It is unnecessary to activate the recombinant LIF.

Sample was diluted partly after adding activating reagent, so please pay attention to this when calculate target protein concentration.

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 μl of the prepared 1x Biotinylated Anti-Mouse Lif 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.

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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.

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-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 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.

Background on Lif

Leukemia inhibitory factor, or LIF, is an interleukin 6 class cytokine that affects cell growth by inhibiting differentiation. When LIF levels drop, the cells differentiate. The LIF was mapped gene to 22q11-q12.2 by Southern analysis of a series of mouse/human somatic cell hybrids and by in situ hybridization to the chromosomes of 2 normal males and some individuals with chromosomal rearrangements. The gene maps between the Philadelphia translocation BCR1 and the breakpoint of the translocation in cell line GM2324 at 22q12.2. LIF derives its name from its ability to induce the terminal differentiation of myeloid leukemic cells, thus preventing their continued growth. Other properties attributed to the cytokine include: the growth promotion and cell differentiation of different types of target cells, influence on bone metabolism, cachexia, neural development, embryogenesis and inflammation.