# LIF ELISA Kit Mouse

# (OKBB00760)

# Instructions for Use

cell culture supernates, serum and plasma (heparin, EDTA)

Variation between lots can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

# Table of Contents

# 1. Background

- **Principle**

  Aviva's mouse LIF ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from rat specific for LIF has been precoated onto 96-well plates. Standards(Expression system for standard: E.coli, Immunogen sequence: S24-F203) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for LIF is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the mouse LIF amount of sample captured in plate.

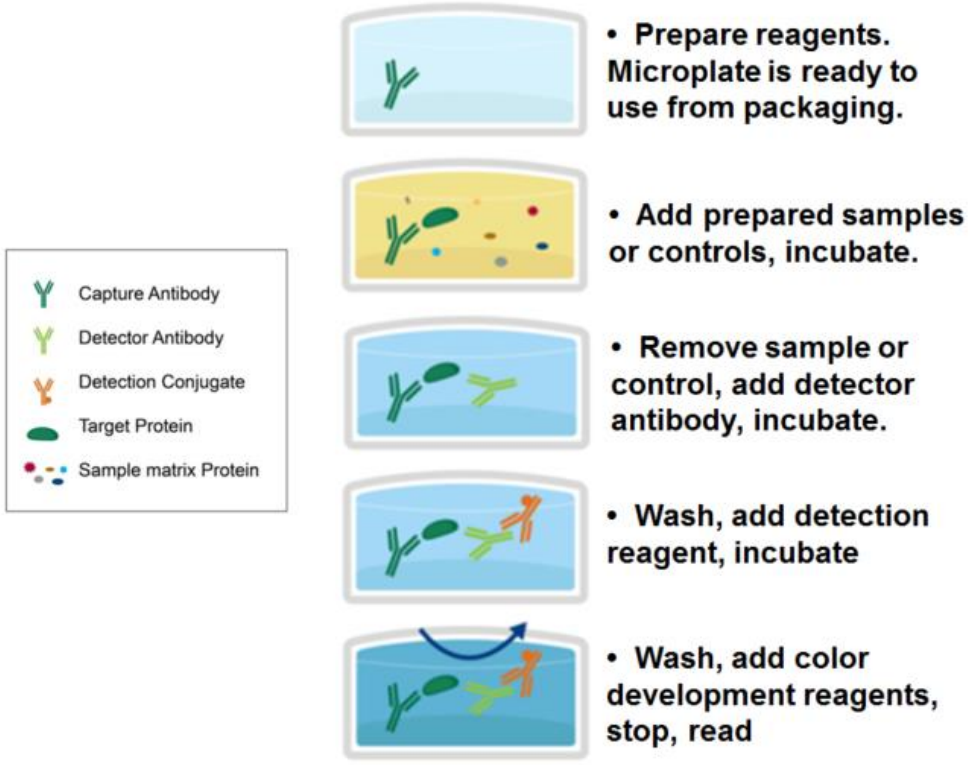
- **Target Background**

  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.

- **General Specifications**

| General Specifications | |
|---|---|
| Range | 7.8pg/ml-500pg/ml |
| LOD | <10pg/ml (Derived by linear regression of $OD_{450}$ of the Mean Blank + 2xSD) |
| Specificity | Mouse LIF<br>UniProt: P09056<br>Gene ID: 16878<br>Target Alias: |
| Cross-Reactivity | No detectable cross-reactivity with other relevant proteins |

## 2. Assay Summary




## 3. Storage and Stability

- Upon receipt store kit at -20°C until expiration date. Avoid multiple freeze/thaw cycles.

## 4. Kit Components

- The following reagents are the provided contents of the kit.

| Description | Quantity | Storage Conditions |
|---|---|---|
| 96-Well Plate Pre-coated with Anti-LIF Antibody | 96 Wells (12 x 8 Well Strips) | Store at -20°C until expiration date |
| Lyophilized Recombinant LIF Standard | 2 x 10 ng | |
| 100X Biotinylated Anti-LIF Antibody | 1 x 130 µL | |
| 100X Avidin-Biotin Peroxidase Complex (ABC) | 1 x 130 µL | |
| Sample Diluent Buffer | 1 x 30 mL | |
| Antibody Diluent Buffer | 1 x 12 mL | |
| ABC Diluent Buffer | 1 x 12 mL | |
| TMB Color Developing Agent | 1 x 10 mL | |
| TMB Stop Solution | 1 x 10 mL | |
| 10X Wash Buffer | 1 x 30 mL | |

## 5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

## 6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.

## 7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception).
- Pipetting less than 1 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
- TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

# 8. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and use immediately.

## 8.1. Standard

8.1.1. Prepare a fresh standard curve for each assay performed. Reconstituted standards cannot be stored for later use. For further directions, please refer to the Certificate of Analysis.

8.1.2. 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.

## 8.2. 1X Biotinylated Anti-Mouse LIF Antibody

8.2.1. Prepare the **1X Biotinylated Mouse LIF Antibody** immediately prior to use by diluting the **100X Biotinylated Mouse LIF Antibody** 1:00 with **Antibody Diluent Buffer**.

8.2.2. For each well strip to be used in the experiment prepare 100 µL by adding 1 µL of **100X Biotinylated Mouse LIF Antibody** to 99 µL **Antibody Diluent Buffer**.

8.2.3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

## 8.3. 1X Avidin-Biotin-Peroxidase Complex (ABC)

8.3.1. Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC) 1:100** with **ABC Dilution Buffer**.

8.3.2. For each well to be used in the experiment prepare 100 µL, by adding 1 µL of **100X Avidin-Biotin-Peroxidase Complex (ABC)** to 99 µL ABC Dilution Buffer.

8.3.3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

### 8.4. 1X Wash Buffer

8.4.1. Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.

8.4.2. Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.

8.4.3. Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4. Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

### 8.5. Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

## 9. Sample Preparation Guidelines

### 9.1. Sample Preparation and Storage

- Samples must be tested to determine if the kit is valid.
- Store samples to be assayed at 4°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

**General Sample Preparation Guidelines**:

- **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 EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Tissue Homogenates -** Rinse 100 mg of tissue with 1X PBS, then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenate for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.
- **Cell Lysates -** Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in PBS (1x) and ultrasonicate the cells 4 times (or freeze cells at ≤ -20°C. Thaw cells

with gentle mixing. Repeat the freeze/thaw cycle 3 times.) Centrifuge at 1,500 x g for 10 minutes at 2-8°C to remove cellular debris.

- **Cell culture supernatants and other biological fluids** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.
- **Saliva** - Collect saliva using a collection device, aliquot and store samples at -20°C. The collection device should not have protein binding or filtering features.
- **Milk -** Centrifuge for 15 min at 1500 x g at 2-8°C. Collect the aqueous layer and repeat this process 3 times. Filter through a 0.2 µm filter. Assay immediately or aliquot and store at -80°C.

### 9.2. Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

- Prior to performing the full experiment, test a serially diluted representative sample.
  - Alternately, a small pool of several samples can also be used with this same method if sample volume is limited.

  Or
  - Refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit
- Dilute samples using **Sample Diluent Buffer**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.

## 10. Assay Procedure

- Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
- Optimal results for intra- and inter-assay reproducibility will be obtained when performing all incubation steps at 37°C as indicated below.

**10.1.** Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.

**10.2.** Cover the plate with the plate sealer and incubate at 37°C for 90 minutes.

**10.3.** Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

**10.4.** Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

**10.5.** Add 100 µL of prepared **1X Biotinylated Anti-Mouse LIF Antibody to each well.**

**10.6.** Cover with the plate sealer and incubate at 37°C for 60 minutes.

**10.7.** Wash plate 3 times with **1X Wash Buffer as** follows:

10.7.1. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.7.2. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.7.3. Add 300 µL of **1X Wash Buffer** to each assay well.

10.7.4. Incubate for 1 minute.

10.7.5. Repeat steps 10.7.1 through 10.7.4 **two** more times.

**10.8.** Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC)** into each well, cover with plate sealer and incubate at 37°C for 30 minutes.

**10.9.** Wash plate 5 times with **1X Wash Buffer as** follows:

10.9.1. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.9.2. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.9.3. Add 300 µL of 1X Wash Buffer to each assay well.

10.9.4. Incubate for 1 minute.

10.9.5. Repeat steps 10.9.1 through 10.9.4 **four** more times.

**10.10.** Add 90 µL of **TMB Color Developing Agent** to each well, cover with plate sealer and incubate at 37°C in the dark for 15-25 minutes.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

**10.11.** Add 100 µL of **TMB Stop Solution** to each well. Well color should change to yellow immediately.

**10.12.** Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.11.

## 11. Calculation of Results

For analysis of the assay results, calculate the **Relative $OD_{450}$** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative $OD_{450}$** of each standard serial dilution point vs. the respective standard concentration. The **LIF** concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative $OD_{450}$** against the standard curve. This is best achieved using curve fitting software. A standard curve should be generated each time the test is performed.

**Note:** if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

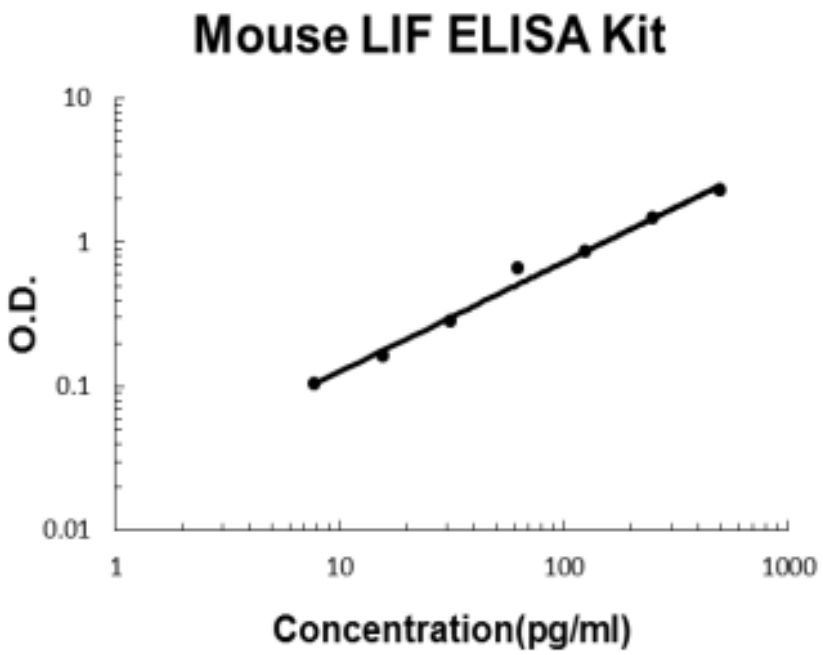
**Note:** if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

## 12. Typical Expected Data

**12.1. Typical absorbance values**. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at $OD_{450}$.

| Standard Number | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|
| Concentration (pg/ml) | 0 | 7.8 | 15.6 | 31.3 | 62.5 | 125 | 250 | 500 |
| $OD_{450}$ | 0.008 | 0.062 | 0.141 | 0.295 | 0.574 | 1.1 | 1.981 | 2.761 |

**12.2. Typical standard curve**. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.




### 12.3. Reproducibility

| | Intra-Assay | | | Inter-Assay | | |
|---|---|---|---|---|---|---|
| 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 |

# 13. Technical Resources

**13.1 Technical Support:**

For optimal service please be prepared to supply the lot number of the kit used.

**USA**

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