# Human LIGHT Pre-Coated ELISA Kit

# Catalog Number: BGK43557

ELISA kit for the accurate quantitation of Human LIGHT concentrations in cell culture supernates, serum, plasma, and other biological fluids.

## Description

LIGHT belongs to the TNF family of ligands, and can signal through the herpes virus entry mediator type A receptor (HVEM, TNFRSF14), LTβR, or bind to a decoy receptor, DcR3. It is expressed in splenocytes, activated PBL, CD8+ tumor infiltrating lymphocytes, granulocytes, and monocytes. LIGHT has the ability to active NF-κB, to co-stimulate the activation of lymphocytes and to induce apoptosis in certain human tumor cells. The human LIGHT gene encodes for a 240 amino acid type II transmembrane protein containing a 37 amino acid cytoplasmic domain, a 21 amino acid transmembrane domain, and a 182 amino acid extracellular domain.

The BioGems Human LIGHT Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human LIGHT with a 96-well strip plate that is pre-coated with mouse monoclonal antibody specific for LIGHT. It contains NS0-expressed recombinant Human LIGHT. The detection antibody is a biotinylated goat polyclonal antibody specific for LIGHT. The kit is analytically validated with ready to use reagents.

## Precaution

For Research use only. Not for use in diagnostic or therapeutic procedures.

Please read the instructions fully prior to beginning the use of the assay kit.

Do not substitute reagents from other sources.

Variations or modifications of the described protocol or the use of other reagents can result in a reduction of product performance.

## Storage and Stability

Upon receipt store the unopened kit at 4°C for 6 months or -20°C for 12 months. Avoid repeated freeze/thaw cycles.

## Materials Provided

| Description | Quantity | Volume |
|---|---|---|
| Anti-Human LIGHT Pre-coated 96-well strip microplate | 1 | 12 strips of 8 wells |
| Human LIGHT Standard | 2 | 10 ng vial |
| Human LIGHT 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 |
| Wash Buffer Concentrate (Powder for 1000mL) | 1 | Pack |
| Color Developing Reagent | 1 | 10mL |
| Stop Solution | 1 | 10mL |
| Plate Sealers | 4 | Each |

## Required Materials not supplied

Microplate Reader capable of reading absorbance at 450nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

Deionized or distilled water.

500mL graduated cylinders.

Test tubes for dilution.

# Reagent Preparation

Bring all reagents to room temperature prior to use.

Wash buffer- Dissolve the included powder in 1000mL of deionized water. Excess wash buffer can be stored for up to one week at 4°C.

Biotinylated Anti-Human LIGHT antibody- It is recommended to prepare this reagent immediately prior to use by diluting the Human LIGHT Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µL by adding 1 µL of Biotinylated antibody (100x) to 99 µL 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 100 µL by adding 1 µL of Avidin-Biotin-Peroxidase Complex (100x) to 99 µL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Human LIGHT Standard- It is recommended that the standards be prepared no greater than 2 hours prior to performing the experiment. Use one 10ng vial of lyophilized Human LIGHT standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10,000 pg/mL using 1mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Dilution of Human LIGHT Standard-

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –2,000pg/mL, #2 –1,000 pg/mL, #3 –500 pg/mL, #4 – 250 pg/mL, #5 –125 pg/mL, #6 –62.5 pg/mL, #7 –31.2 pg/mL, #8 – 0.0 (Blank).
2. To generate standard #1, add 200 µL of the reconstituted standard stock solution of 10,000 pg/mL and 800 µL of sample diluent to tube #1 for a final volume of 1,000 µL. 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.

Microplate- The included microplate is provided ready to use and does not require additional rinsing or blocking. The unused well strips should be sealed and stored in the original packaging.

## Sample Preparation

The recommended sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

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. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.

## 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 procedure

It is recommended that all reagents and materials be equilibrated to room temperature prior to the experiment.

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. 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-Human Growth Hormone 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 more additional times.
9. Add 100 µL of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 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 4 more additional times.
11. Add 90 µL of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 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.

# Calculation of results

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.

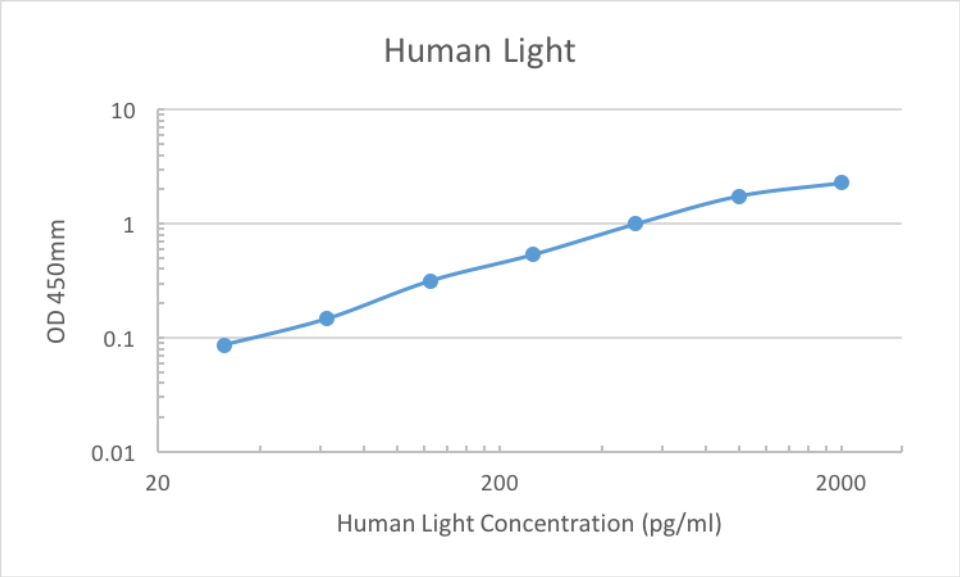
For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

# Typical Data

| **Standard #** | **8** | **7** | **6** | **5** | **4** | **3** | **2** | **1** |
|---|---|---|---|---|---|---|---|---|
| Standard Concentration (pg/mL) | 0 | 31.2 | 62.5 | 125 | 250 | 500 | 1000 | 2000 |
| $OD_{450}$ | 0.006 | 0.086 | 0.146 | 0.313 | 0.533 | 0.993 | 1.745 | 2.284 |

## Typical Standard Curve

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




## Sensitivity

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.

## Specifications

Range- 31.2pg/ml-2000pg/ml

Sensitivity- <10pg/ml

Specificity- Natural and recombinant Human LIGHT (UniProt ID: O43557)

Cross-Reactivity- No detectable cross-reactivity with other relevant proteins

## Reproducibility

| | Intra-Assay | | | Inter-Assay | | |
|---|---|---|---|---|---|---|
| **Sample ID** | 1 | 2 | 3 | 1 | 2 | 3 |
| n= | 16 | 16 | 16 | 24 | 24 | 24 |
| Mean Measured Concentration (pg/mL) | 323 | 853 | 1285 | 354 | 887 | 1366 |
| Standard Deviation | 13.6 | 43.5 | 46.3 | 27.6 | 67.4 | 97 |
| Consistency (%CV) | 4.2 | 5.1 | 3.6 | 7.8 | 7.6 | 7.1 |