Quantikine® ELISA

Human LIGHT/TNFSF14 Immunoassay

Catalog Number DLIT00

For the quantitative determination of human LIGHT concentrations in cell culture supernates, serum, plasma, and saliva.

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

LIGHT (homologous to lymphotoxins, exhibits inducible expression, and competes with HSV glycoprotein D for HVEM, a receptor expressed by T lymphocytes, also reported as HVEM Ligand and LT-γ) is a member of the tumor necrosis factor superfamily (TNFSF) designated TNFSF14. Originally reported in 1998 (1-3), LIGHT is a type II transmembrane protein expressed on activated T cells and immature dendritic cells (4, 5). LIGHT signals via the lymphotoxin β receptor (LTβR) and the herpes virus entry mediator (HVEM) and may be regulated by the binding of decoy receptor 3 (DcR3, also known as TR6) (1-3, 6). LIGHT has been implicated in T cell development and homeostasis, dendritic cell maturation, and atherogenesis (4, 7-9). LIGHT may play a prominent role in diseases involving T cells such as graft versus host disease (GVHD) and autoimmune conditions and may also function to block viral entry (10, 11). For a commentary on LIGHT, see reference 12.

LIGHT is a 240 amino acid (aa) protein consisting of a 37 aa cytoplasmic domain, a 22 aa transmembrane region, and a 181 aa extracellular domain. The mouse homologue of human LIGHT is 77% identical in primary structure (10, 13). A splice variant of human LIGHT (missing 36 internal amino acids) has also been observed but appears not to be secreted (11). In addition, MMP-mediated membrane shedding of LIGHT results in an approximately 26 kDa soluble ligand capable of binding HVEM (11). Similar to other TNFSF members, LIGHT is predicted to assemble and function as a homotrimer (3, 14). The LIGHT gene maps to chromosome 19 (11).

LIGHT mRNA has been detected in activated PBMCs, lymph nodes, spleen, heart, and lung tissues but is absent in most tumor-derived cell lines (1, 2, 15). Experiments using HVEM/Fc chimera, LTβR/Fc chimera, and/or antibodies against LIGHT reveal expression on the surface of immature, but not mature, dendritic cells (DCs) (4). It is expressed on activated, but not resting, T-lymphocytes and appears to feature more prominently on the $CD8^+$, or cytotoxic T lymphocyte (CTL) subset (5). There is evidence that LIGHT down-regulates HVEM expression (5). The LIGHT receptor HVEM is expressed predominantly on lymphoid cells, whereas the alternative LIGHT receptor, LTβR, is expressed on stromal and epithelial cells, but is conspicuously absent on lymphocytes (1, 5, 6). Transcript for the LIGHT receptor DcR3, which is devoid of signaling machinery, is expressed at high levels in many normal human tissues such as stomach, spinal cord, colon, lymph node, and spleen, but is expressed much more weakly in thymus and peripheral blood lymphocytes (16).

LIGHT may function in promoting the maturation of DCs, activation and expansion of T cells, and the apoptotic negative selection of autoreactive thymocytes (4, 7, 8, 17, 18). LIGHT is expressed on the surface of immature DCs, and cooperates with CD40 Ligand (CD154) to induce DC maturation (4). Additionally, LIGHT enhances the antigen-dependent activation and expansion of T cells, mediated by DCs and other antigen presenting cells (APCs), and appears to be important in communication between APCs and T cells (4, 18). LIGHT-induced T cell expansion can also proceed through an APC-independent, T cell to T cell interaction (17). Two lines of transgenic (Tg) mice that constitutively express LIGHT in the T cell lineages have recently been established (7, 17). Both lines develop severe autoimmune disease manifested by profound inflammation caused by activated T cells and lymphoid tissue abnormalities such as splenomegaly and lymphadenopathy (7, 17). Blockade of LIGHT in one Tg model lessened the severity of the induced condition (17) and has been shown to prevent colitis in a T cell-dependent transfer model (19). It is unclear at this time whether LIGHT Tg mice suffer from non-specific inflammation caused by overactive T lymphocytes or from loss of tolerance to self-antigens. LIGHT is able to induce apoptosis in some tumor cell lines, an effect that is enhanced by Bcl-2 over-expression (14, 15). The thymi of LIGHT Tg mice are atrophied and contain low numbers of $CD4^+$ $CD8^+$ T cells, and blockade of LIGHT in a TCR Tg model results in the rescue of auto-antigen-stimulated T cells (8). These observations suggest a role for LIGHT in thymic negative selection. Overall, LIGHT appears to have multiple, sometimes contrasting functions. Attempts to isolate cause and effect relationships are complicated by the communal receptor usage among TNFSF members and the differential expression of TNFSF receptors.

The Quantikine Human LIGHT Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human LIGHT in cell culture supernates, serum, plasma, and saliva. It contains NS0-expressed recombinant human LIGHT and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human LIGHT showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human LIGHT.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human LIGHT has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any LIGHT present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human LIGHT 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 LIGHT 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.
- Samples, controls, and standards must be pipetted within 15 minutes.
- 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.

<table>
<tr><th>PART</th><th>PART #</th><th>DESCRIPTION</th><th>STORAGE OF OPENED/ RECONSTITUTED MATERIAL</th></tr>
<tr><td>Human LIGHT Microplate</td><td>892147</td><td>96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human LIGHT.</td><td>Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Human LIGHT Standard</td><td>892149</td><td>Recombinant human LIGHT in a buffer with preservatives; lyophilized. Refer to the vial label for reconstitution volume.</td><td>Aliquot and freeze at ≤ -20 °C in a manual defrost freezer for up to 1 month.* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Human LIGHT Conjugate</td><td>892148</td><td>21 mL of polyclonal antibody specific for human LIGHT conjugated to horseradish peroxidase with preservatives.</td><td rowspan="8">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1W</td><td>895117</td><td>11 mL of a buffered protein base with preservatives.</td></tr>
<tr><td>Calibrator Diluent RD5K</td><td>895119</td><td>21 mL of a buffered protein base with preservatives. For cell culture supernate and saliva samples.</td></tr>
<tr><td>Calibrator Diluent RD6-6</td><td>895177</td><td>21 mL of a buffered protein base with preservatives. For serum and plasma samples.</td></tr>
<tr><td>Wash Buffer Concentrate</td><td>895003</td><td>21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives. May turn yellow over time.</td></tr>
<tr><td>Color Reagent A</td><td>895000</td><td>12 mL of stabilized hydrogen peroxide.</td></tr>
<tr><td>Color Reagent B</td><td>895001</td><td>12 mL of stabilized chromogen (tetramethylbenzidine).</td></tr>
<tr><td>Stop Solution</td><td>895032</td><td>6 mL of 2 N sulfuric acid.</td></tr>
<tr><td>Plate Sealers</td><td>N/A</td><td colspan="2">4 adhesive strips.</td></tr>
</table>

* 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.
- Collection device for saliva samples which has no enzyme binding or filtering capabilities such as a Salivette® or equivalent.
- Test tubes for dilution of standards and samples.
- Human LIGHT Controls (optional; R&D Systems, Catalog # QC114).

PRECAUTIONS

LIGHT is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 at room temperature 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.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector cannot have any enzyme binding or filtering capabilities.*

SAMPLE PREPARATION

Saliva samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5K.

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REAGENT PREPARATION

Bring all reagents to room temperature before use.

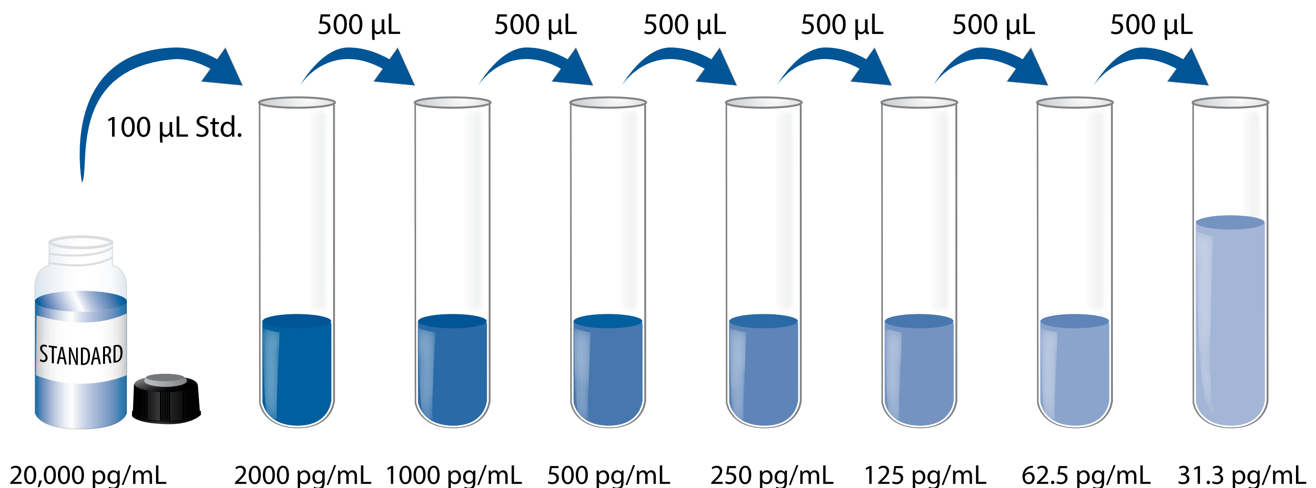
Note: *High concentrations of LIGHT are found in saliva. Using a face mask and gloves is recommended to protect kit reagents from contamination.*

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 LIGHT Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human LIGHT Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μL of Calibrator Diluent RD5K (*for cell culture supernate and saliva samples*) or Calibrator Diluent RD6-6 (*for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 500 μL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. 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 samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples 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. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.

   Note: *Samples must be pipetted within 15 minutes.*
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 (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 LIGHT Conjugate to each well. Cover with a new adhesive strip. 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. **Protect from light.**
   For cell culture supernate/saliva samples: Incubate for **20 minutes** at room temperature.
   For serum and plasma samples: Incubate for **30 minutes** at room temperature.
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 well is green or if 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.

*Samples may require dilution. See Sample Preparation.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

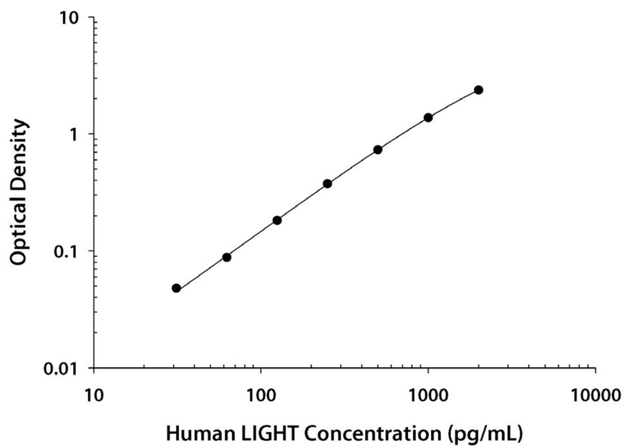
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

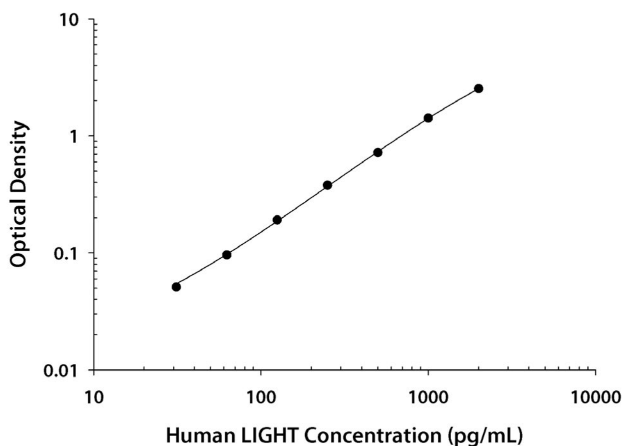
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATOR DILUENT RD5K



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.022	0.022	—
31.3	0.068 0.072	0.070	0.048
62.5	0.108 0.111	0.110	0.088
125	0.203 0.205	0.204	0.182
250	0.384 0.408	0.396	0.374
500	0.754 0.756	0.755	0.733
1000	1.378 1.410	1.394	1.372
2000	2.382 2.406	2.394	2.372

CALIBRATOR DILUENT RD6-6



(pg/mL)	O.D.	Average	Corrected
0	0.032 0.034	0.033	—
31.3	0.080 0.087	0.084	0.051
62.5	0.126 0.131	0.129	0.096
125	0.215 0.233	0.224	0.191
250	0.406 0.418	0.412	0.379
500	0.744 0.764	0.754	0.721
1000	1.424 1.475	1.450	1.417
2000	2.521 2.601	2.561	2.528

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/SALIVA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	308	626	1181	318	616	1177
Standard deviation	11.7	25.3	34.6	27.0	44.2	81.8
CV (%)	3.8	4.0	2.9	8.5	7.2	6.9

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	350	712	1388	387	754	1469
Standard deviation	12.0	31.7	63.3	19.6	31.8	63.4
CV (%)	3.4	4.5	4.6	5.1	4.2	4.3

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	105	100-113%
Serum (n=5)	102	85-114%
EDTA plasma (n=5)	106	91-114%
Heparin plasma (n=5)	101	89-115%

SENSITIVITY

Eighty-one assays were evaluated and the minimum detectable dose (MDD) of human LIGHT ranged from 1.2-16.5 pg/mL. The mean MDD was 5.5 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.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human LIGHT were serially diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Saliva (n=2)
1:2	Average % of Expected	104	101	97	98	92
	Range (%)	97-108	96-107	90-103	95-100	89-94
1:4	Average % of Expected	104	99	97	96	86
	Range (%)	98-110	91-107	91-105	89-101	——
1:8	Average % of Expected	103	104	95	98	——
	Range (%)	92-109	93-115	87-115	92-107	——
1:16	Average % of Expected	106	100	97	96	——
	Range (%)	100-113	88-112	88-103	87-109	——

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human LIGHT produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Saliva - Samples from apparently healthy volunteers were evaluated for the presence of human LIGHT in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=60)	112	93	ND-216
EDTA plasma (n=35)	——	0	ND
Heparin plasma (n=35)	42	7	ND-55.6
Saliva (n=12)	147	50	ND-411

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human LIGHT.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	249
Stimulated	242	794

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human LIGHT.

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

Recombinant human:

ANG
Ang-2
AR
BDNF
CD4
CD40
CD40 Ligand
CNTF
CT-1
CTLA-4
Epo
Fas
GDNF
GITR
GITR Ligand
HVEM
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-12 p40
IL-13
IL-15
IL-16
IL-17
Leptin
LIF
MIF
MK
NT-3
NT-4
OPG
OSM
PTN
SCF
SLPI
SMDF
TNF-α
TNF-β
Tpo
TRAIL
TRANCE

Recombinant mouse:

CT-1
CTLA-4
Fas
Fas Ligand
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-11
IL-12
IL-12 p40
IL-13
IL-17
Leptin
LIF
OPG
OPN
OSM
SCF
TNF-α
Tpo
TRANCE

Recombinant rat:

CNTF
GDNF
IFN-γ
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-10
Leptin
TNF-α

Recombinant porcine:

IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-8
IL-10
TNF-α

Recombinant human DcR3/Fc Chimera interferes at concentrations ≥ 500 pg/mL

For research use only. Not for use in diagnostic procedures.

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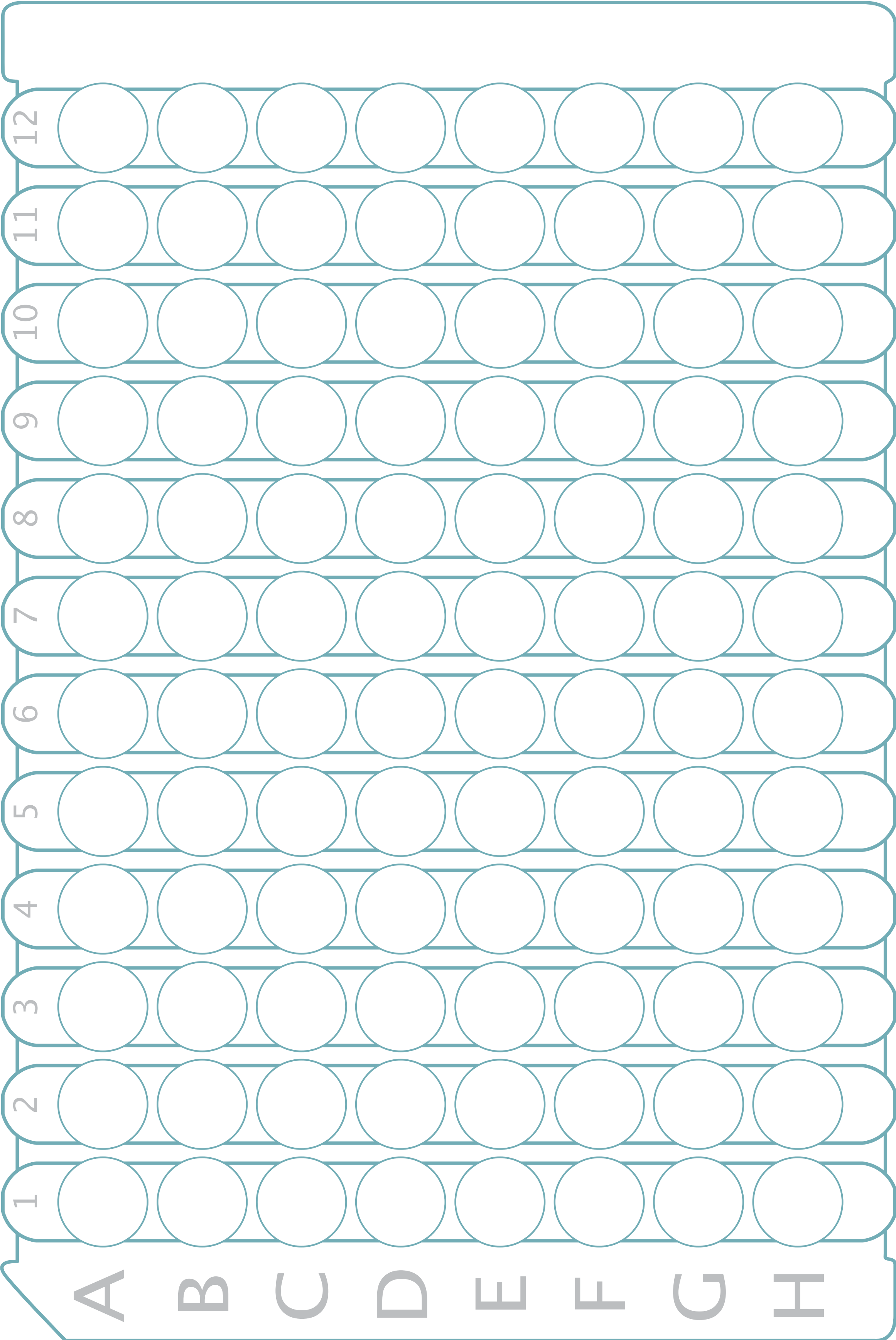
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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NOTES

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