Manual
LIGHT, Soluble (human) Detection Set
(for ELISA Application)

[TNFSF 14, Soluble (human) Detection Set]

For Research Use Only

APO-54N-029
Version 1 (26-Nov-07)
1. INTENDED USE

The LIGHT, Soluble (human) Detection Set is to be used for the **in vitro** quantitative determination of soluble human LIGHT in cell supernatant, plasma and serum. This Detection Set is for research use only.

2. INTRODUCTION

LIGHT (**derived from “homologous to Lymphotoxins, exhibits Inducible expression, and competes with Herpes simplex virus (HSV) G**lycoprotein D for **H**erpes virus entry **m**ediator (HVEM /TR2), a receptor expressed by **T** lymphocytes**) is a 29 kDa type 2 transmembrane protein that belongs to the TNF superfamily [1, 2]. It is expressed in granulocytes, monocytes, platelets, immature dendritic cells and activated T cells. LIGHT is active either as membrane-anchored or secreted (soluble) forms. Three receptors of the TNF superfamily: lymphotoxin receptor (LTR), herpesvirus entry mediator (HVEM), and decoy receptor 3 (DcR3) binds to the ligand. LIGHT is involved in T-cell regulation and dendritic cell maturation [3]. It triggers several signaling pathways such as NF-κB, non-canonical NF-κB, Stat3, ERK5 / BMK1 and JNK [4]. LIGHT is released from platelets and induces a proinflammatory state in vascular endothelial cells [5]. It suppress tumor formation in vivo by inducing apoptotic cell death. Embryonic stem (ES) cells are differentiated in the presence of LIGHT [6]. Mice overexpressing LIGHT spontaneously develop severe autoimmune disease. LIGHT has been reported to increase in Rhumatoid Arthritis (RA) patients and to induce a RANKL - dependent and -independent osteoclasts formation [7].

3. PRINCIPLE OF PROCEDURE

This assay is a sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) developed for the direct measurement of human LIGHT (hLIGHT) in biological fluids. A monoclonal antibody specific for hLIGHT (COAT) is coated onto the wells of the microtiter plate. Samples and standards of hLIGHT are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, hLIGHT is recognized by the addition of a biotinylated monoclonal antibody specific for hLIGHT (DET). After removal of excess biotinylated antibody, streptavidine-peroxidase is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3′,5,5′-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of hLIGHT in the samples.

4. MATERIALS PROVIDED

- 1 vial Standard (lyophilized) (1 µg) (STD) APO-54N-029/STD
- 1 vial Coating Antibody (120µl) (COAT) APO-54N-029/COAT
- 1 vial Detection Antibody (120µl) (DET) APO-54N-029/DET

5. MATERIALS REQUIRED

- PBS: 137mM NaCl, 2.7mM KCl, 8.1mM Na₂HPO₄, 1.5mM KH₂PO₄, pH 7.2, 0.2µM filtered
- Wash Buffer: 0.1% Tween® 20 in PBS
- Diluent Buffer: 2% BSA in PBS, 0.2µM filtered.
- ELISA Buffer: 0.2% BSA and 0.05% Tween® 20 in PBS
- 2M sulphuric acid (H₂SO₄)
- Tetramethylbenzidine substrate kit (SeraCare Life Sciences, code 91000-01K)
- Streptavidin-peroxidase (Jackson immunoresearch, N° 016-030-084)
- HPLC grade water
- Nunc MaxiSorpTM flat-bottom 96 well plate

6. PRODUCT SPECIFICATION

**Number of Assays:** This Detection Set contains sufficient materials to run ELISAs on 5 x 96-well plates.

**Specificity:** The antibodies used in this detection Set are specific for measurement of natural and recombinant human LIGHT.

**Sensitivity:** 0.2ng/ml (range 0 to 20 ng /ml)

**Stability:** Stable at least 6 months after receipt when stored at +4°C. For long term storage, keep the standard (STD) at -20°C
7. GENERAL ELISA PROTOCOL

PREPARATION OF REAGENTS

1. Dilute the desired amount of Coating Antibody (COAT) (1mg/ml) to 2µg/ml in PBS without carrier protein.
2. Dilute the desired amount of Detection Antibody (DET) (1mg/ml) to 2µg/ml in Diluent Buffer.
3. Reconstitute the Standard Protein (STD) with 50µl PBS to obtain a concentration of 0.02 mg/ml. **After reconstitution, prepare aliquots and store the reconstituted standard at -20°C! Avoid freeze/thaw cycles!** A standard curve using 2-fold serial dilutions in ELISA buffer is recommended. Suggested standard points are 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312ng/ml and 0 ng/ml.
4. If measuring plasma, serum or cell culture supernatant, dilute samples in ELISA buffer.
5. To reduce interference from rheumatoid factor in the serum, Ig can be cleared by treating serum dilutions with 0.05 volume of protein G-Sepharose twice for one hour at 4°C before pelleting the beads and collecting supernatants.

PLATE PREPARATION

1. Coat the wells by adding 100µl/well of diluted (2 µg/ml) Coating Antibody (COAT) to a 96-well ELISA microplate (Nunc MaxiSorp™ flat-bottom 96 well plate is suggested). Cover the plate with plastic film and leave 2 hours at 37°C.
2. Aspirate the coated wells and add 300µl of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300µl of Diluent Buffer at 37°C for 1 hour.
4. Repeat the aspiration/wash as in step 2 for a total of five washes.
Assay Procedure

1. Add a total of 100µl/well diluted plasma, serum, culture supernatant or Standard Protein serial dilutions in ELISA Buffer to the plate.
2. Cover the plate with plastic film and incubate for 2 hours at RT°C.
3. Aspirate the coated wells and add 300µl of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
4. Add 100µl/well of the diluted (2µg/ml) Detection Antibody (DET).
5. Cover the plate with plastic film and incubate for one hour at RT°C.
6. Repeat the aspiration/wash as in step 3 for a total of five washes.
7. Add 100 µl to each well of the diluted HRP Labelled Streptavidin (from Jackson immunoresearch, dilute 1/5'000).
8. Cover the plate with plastic film and incubate for 30 min at RT°C.
9. Aspirate the coated wells and add 300 µl of Wash Buffer (Wash Buffer 1X) using a multichannel pipette or autowasher. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
10. Substrate development is conducted by addition of 100µl to each well of ready-to-use tetramethylbenzidine (TMB) (SeraCare Life Sciences) for 20-30 min.
11. Stop the reaction by adding 50µl of 2M H₂SO₄. Tap the plate gently to ensure thorough mixing.
12. Measure the OD at 450nm in an ELISA reader.
13. Measure absorbance at 550nm and subtract these values from those obtained at 450nm to correct for optical imperfections in the microplate. If absorbance at 550nm is not possible, measure the absorbance at 450nm only.

Note: When the 550 nm measurement is omitted, absorbance values will be higher.
8. TECHNICAL HINTS AND LIMITATIONS

• Once reagents have been added to the plate, DO NOT let the plate dry at any time during the assay.
• Be sure no sodium azide is present in this assay, as this inhibits HRP enzyme activity.
• It is recommended that all standards and samples be assayed in duplicate.
• Avoid microbial contamination of reagents and equipment. Automated plate washers can become contaminated thereby causing assay variability. Buffers containing a large quantity of protein should be made under sterile conditions and stored at 2-8°C or be prepared fresh daily.
• Vigorous plate washing is essential.
• Avoid exposing reagents to excessive heat or light during storage and incubation.
• Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedure.

9. CALCULATION OF RESULTS

• Average the duplicate readings for each standard, control and sample and subtract the average blank value.
• Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding hLIGHT concentration (ng/ml) on the horizontal axis (see TYPICAL DATA). Calculate results using graph paper or curve-fitting statistical software. The amount of hLIGHT in each sample is determined by interpolating for the absorbance value (Y axis) using the standard curve.
• If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate ng/ml of human LIGHT in the sample.
10. TYPICAL DATA

The following data are obtained using the different concentrations of standard as described in this protocol:

<table>
<thead>
<tr>
<th>Standard hLIGHT (ng/ml)</th>
<th>Optical Density (mean)</th>
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<tbody>
<tr>
<td>0</td>
<td>0.116</td>
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<tr>
<td>0.312</td>
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Figure: Standard curve
Europe
Tel: +41 61 926 89 99
Fax: +41 61 926 89 95
orders@apotech.com

North America
Tel: (858) 550-8828
Fax: (858) 550-8825
apotechus@apotech.com

www.apotech.com