Product Manual

Human LRP1 ELISA Kit

Catalog Number
STA-609  96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures
Introduction
LDL Receptor-Related Protein 1 (LRP1), also known as alpha-2-macroglobulin receptor (A2MR), apolipoprotein E receptor (APOER) or cluster of differentiation 91 (CD91), is a member of the LDL receptor family (Figure 1). LRP1 is ubiquitously expressed, binds to over 40 different ligands, and functions in a diverse set of biological processes.

Figure 1. LDL receptor family members and structures. A schematic diagram illustrates the 5 domains of the LDL receptor family: 1) LDLR type A repeat domains (green); 2) epidermal growth factor (EGF) receptor homology domain containing the β-propeller subdomain (cyan); 3) O-linked glycosylation domain (gold); 4) transmembrane domain (red); 5) cytoplasmic domain containing NPXY sequence (blue).

LRP1 is expressed abundantly in the liver where it recognizes and serves to clear a variety of circulatory molecules such as proteinase-inhibitor complexes, serpin enzyme complexes (SECs), chylomicron remnants, as well as activated coagulation factors. In smooth muscle cells of the arterial wall, LRP1 directly or indirectly down regulates the Platelet Derived Growth Factor (PDGF) signaling pathway, thereby antagonizing smooth muscle cell proliferation/migration which can lead to atherosclerosis. LRP1 is also a transforming growth factor beta (TGF-β) receptor, and is required to mediate the growth inhibition effects of TGF-β. Additionally, LRP1 expression in macrophages has been shown to confer an atheroprotective effect, although the specific mechanism has not been defined. LRP1 is also highly expressed in adipocytes where it is thought to mediate the endocytic clearance of triglycerides. In the brain, LRP1 helps maintain blood brain barrier permeability through
the activity of tissue-type plasminogen activator (tPA). Furthermore, LRP1 mediates the internalization of amyloid precursor protein (APP), which leads to β-amyloid peptide production, an event that is central to the development of Alzheimer’s Disease.

Through direct interaction with integrins, or by modulating the activity of other proteins, LRP1 can affect the process of cell migration. A complex formed with LR1P1, calreticulin (CRT), and the integrin binding protein thrombospondin-1 (TSP-1) leads to large G protein-coupled signaling and stimulation of cell migration. Although this type of signaling is usually observed through 7-transmembrane G protein-coupled receptors, LR1P1 signaling through G proteins has been observed in other tissues. For example, in neurons apolipoprotein E4 can trigger apoptosis through LR1P1 to G protein-coupled signaling. In addition to participating in integrin signaling, LR1P1 assists in β1-integrin trafficking from the endoplasmic reticulum to the cell surface.

LR1P1 also plays a role in macrophage phagocytosis. It has been shown that LR1P1 complexed with CRT enhances phagocytosis of apoptotic cells that have been marked for ingestion with C1q and mannose binding lectin (MBL).

Finally, LR1P1 has been implicated in regulating immune responses. LR1P1 binds alpha-2-macroglobulin (α2M). In a study using special T hybridomas that only respond to hen egg lysozyme in a MHC-restricted fashion, when non-covalent complexes of lysozyme-α2M-elastase were incubated with macrophages, over 200 fold less antigen was required compared to free lysozyme incubation to achieve detectable presentation to the T hybridomas. This result suggests that LR1P1-competent forms of α2M can upregulate the processing of antigen through an LR1P1 mediated mechanism.

Cell Biolabs’ Human LR1P1 Kit is an enzyme immunoassay developed for the detection and quantitation of human LR1P1 in plasma, serum, cell or tissue lysate samples. LR1P1 is captured on a plate that is coated with LDL receptor associated protein (LRPAP, also known as receptor associated protein or RAP). LR1P1 is then detected with an anti-LR1P1 antibody followed by an HRP conjugated secondary antibody. The kit has a detection sensitivity limit of 50 ng/mL human LR1P1. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

**Related Products**

1. STA-368: Human ApoB ELISA Kit
2. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
3. STA-386: LDL Receptor ELISA Kit
4. STA-387: Human LOX-1 ELISA Kit
5. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
6. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)
Kit Components

**Box 1 (shipped at room temperature)**
1. 96-well Protein Binding Plate (Part No. 231001): One 96-well strip plate.
2. Anti-Human LRP1 Antibody (1000X) (Part No. 260902): One 10 μL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part No. 310808): One 12 mL bottle.

**Box 2 (shipped on blue ice packs)**
1. Human LRP1 Standard (Part No. 260903): One 1.25 mL vial of 6 μg/mL Human LRP1 containing 1% Triton X-100, 1 mM PMSF and HSA as carrier protein.
2. Recombinant LRPAP (Part No. 260901): One 200 μL vial at 0.5 mg/mL.

**Materials Not Supplied**
1. Plasma, Serum, Cell or Tissue Lysate
2. PBS containing 0.1% BSA
3. 10 μL to 1000 μL adjustable single channel micropipettes with disposable tips
4. 50 μL to 300 μL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

**Storage**
Upon receipt, aliquot and store the Human LRP1 Standard at -20°C and store the Recombinant LRPAP at -80°C. Avoid multiple freeze/thaw cycles. Store all other components at 4°C.

**Preparation of Reagents**
- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-Human LRP1 Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute the Anti-Human LRP1 Antibody or the Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.
- LRPAP Coated Plate: Dilute the proper amount of Recombinant LRPAP (0.5 mg/mL) to 10 μg/mL in 1X PBS. For example, add 20 μL of Recombinant LRPAP to 980 μL of PBS. Add 100 μL of the 10 μg/mL Recombinant LRPAP to each well and incubate overnight at 4°C.
Remove the Recombinant LRPAP coating solution and wash once with dH2O. Blot plate on paper towels to remove excess fluid. Add 200 μL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4°C and remove the Assay Diluent immediately before use.

*Note: The Recombinant LRPAP coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.*

**Preparation of Human LRP1 Standard**

Prepare a dilution series of human LRP1 standard in the concentration range of 0 to 3 μg/mL in Assay Diluent (Table 1).

<table>
<thead>
<tr>
<th>Standard Tubes</th>
<th>6 μg/mL Human LRP1 Standard (μL)</th>
<th>Assay Diluent (μL)</th>
<th>Human LRP1 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>250</td>
<td>3000</td>
</tr>
<tr>
<td>2</td>
<td>250 of Tube #1</td>
<td>250</td>
<td>1500</td>
</tr>
<tr>
<td>3</td>
<td>250 of Tube #2</td>
<td>250</td>
<td>750</td>
</tr>
<tr>
<td>4</td>
<td>250 of Tube #3</td>
<td>250</td>
<td>375</td>
</tr>
<tr>
<td>5</td>
<td>250 of Tube #4</td>
<td>250</td>
<td>188</td>
</tr>
<tr>
<td>6</td>
<td>250 of Tube #5</td>
<td>250</td>
<td>93.8</td>
</tr>
<tr>
<td>7</td>
<td>250 of Tube #6</td>
<td>250</td>
<td>46.9</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>250</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Preparation of Human LRP1 Standards

**Preparation of Samples**

The following recommendations are only guidelines and may be altered to optimize or complement the user’s experimental design.

- **Plasma:** Collect blood with heparin, EDTA, or citrate and centrifuge for 10 minutes at 1000 x g at 4°C. Remove the plasma and assay immediately or store samples at -80°C for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- **Serum:** Collect blood and allow to clot at room temperature. Centrifuge for 10 minutes at 1000 x g at 4°C. Assay immediately or store samples at -80°C for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- **Cell or Tissue Lysate:** Sonicate or homogenize sample with cold 1XPBS containing 1% Triton X-100 or 1% NP40 and centrifuge at 10,000 x g for 10 minutes at 4°C. Assay immediately or store samples at -80°C for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

*Note: RIPA buffer should not be used because it inhibits the assay due to the presence of sodium deoxycholate.*

**Assay Protocol**

1. Add 100 μL of human LRP1 unknown sample or standard to the LRPAP Coated Plate. Each human LRP-1 unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at 37°C for at least 2 hours or 4°C overnight.

3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

4. Add 100 µL of the diluted Anti-Human LRP1 Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.

5. Wash the strip wells 3 times according to step 3 above.

6. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.

7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.

8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength.

**Example of Results**
The following figures demonstrate typical results with the Human LRP1 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.
Figure 2: Human LRP1 ELISA Standard Curve.

Figure 3: Detection of soluble LRP1 in human plasma.
References

Warranty
These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS’ sole obligation and purchaser’s exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information
Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2014: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.