Product Manual

Human LRP1 ELISA Kit

Catalog Number

STA-609 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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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.

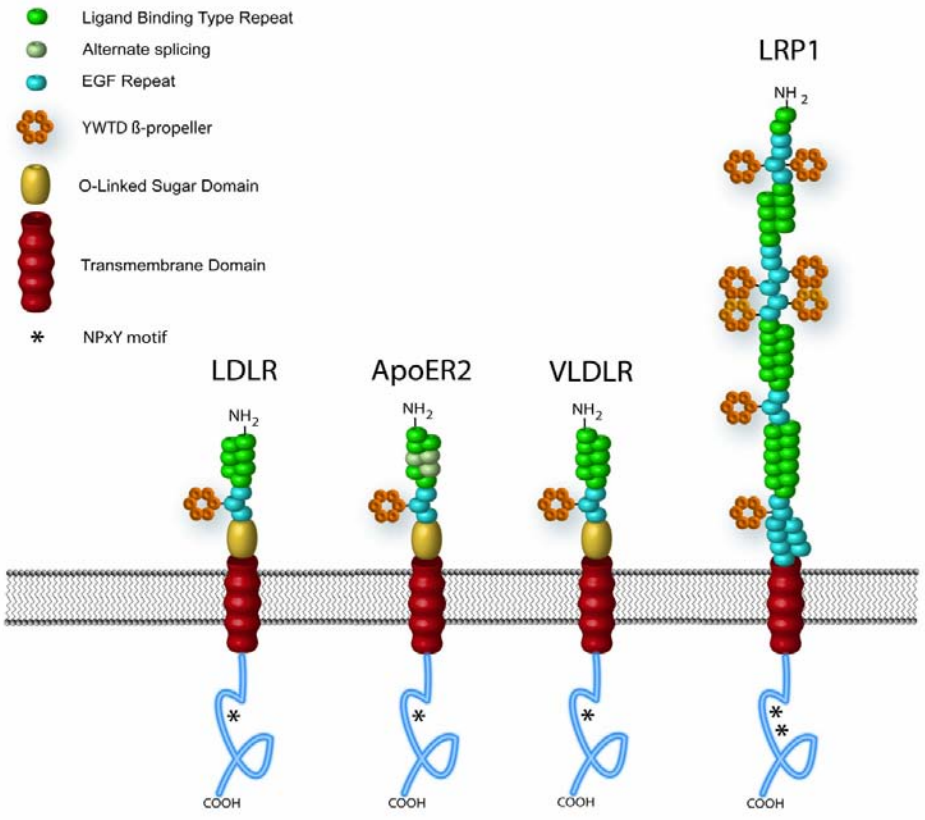

Figure 1. LDL receptor family members and structures. A schematic diagram illustrates the 5 domains of the LDL receptor family: 1) LDLR type A repeat domains (green); 2) epidermal growth factor (EGF) receptor homology domain containing the β-propeller subdomain (cyan); 3) O-linked glycosylation domain (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 LRP1, 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, LRP1 signaling through G proteins has been observed in other tissues. For example, in neurons apolipoprotein E4 can trigger apoptosis through LRP1 to G protein-coupled signaling. In addition to participating in integrin signaling, LRP1 assists in β1-integrin trafficking from the endoplasmic reticulum to the cell surface.

LRP1 also plays a role in macrophage phagocytosis. It has been shown that LRP1 complexed with CRT enhances phagocytosis of apoptotic cells that have been marked for ingestion with C1q and mannose binding lectin (MBL).

Finally, LRP1 has been implicated in regulating immune responses. LRP1 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 LRP1-competent forms of α2M can upregulate the processing of antigen through an LRP1 mediated mechanism.

Cell Biolabs' Human LRP1 Kit is an enzyme immunoassay developed for the detection and quantitation of human LRP1 in plasma, serum, cell or tissue lysate samples. LRP1 is captured on a plate that is coated with LDL receptor associated protein (LRPAP, also known as receptor associated protein or RAP). LRP1 is then detected with an anti-LRP1 antibody followed by an HRP conjugated secondary antibody. The kit has a detection sensitivity limit of 50 ng/mL human LRP1. 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.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 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 -20ºC and store the Recombinant LRPAP at -80ºC. Avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

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 4ºC.

Remove the Recombinant LRPAP coating solution and wash once with dH_2O. 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 4ºC 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).

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

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 4ºC. Remove the plasma and assay immediately or store samples at -80ºC 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 4ºC. Assay immediately or store samples at -80ºC 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 4ºC. Assay immediately or store samples at -80ºC 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 4ºC 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 wave length.

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.

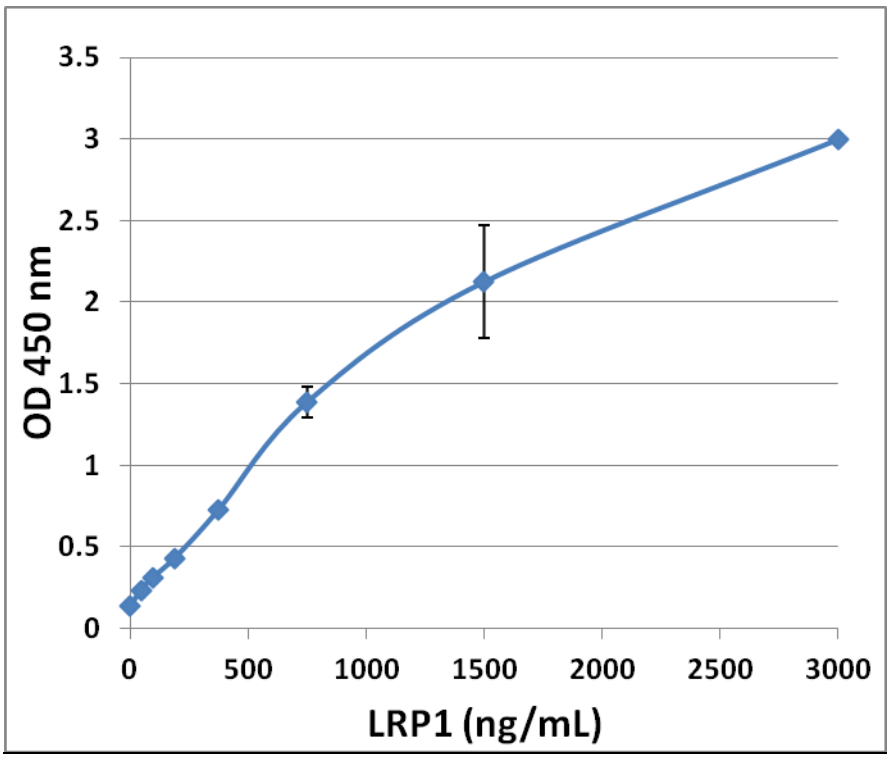

Figure 2: Human LRP1 ELISA Standard Curve.

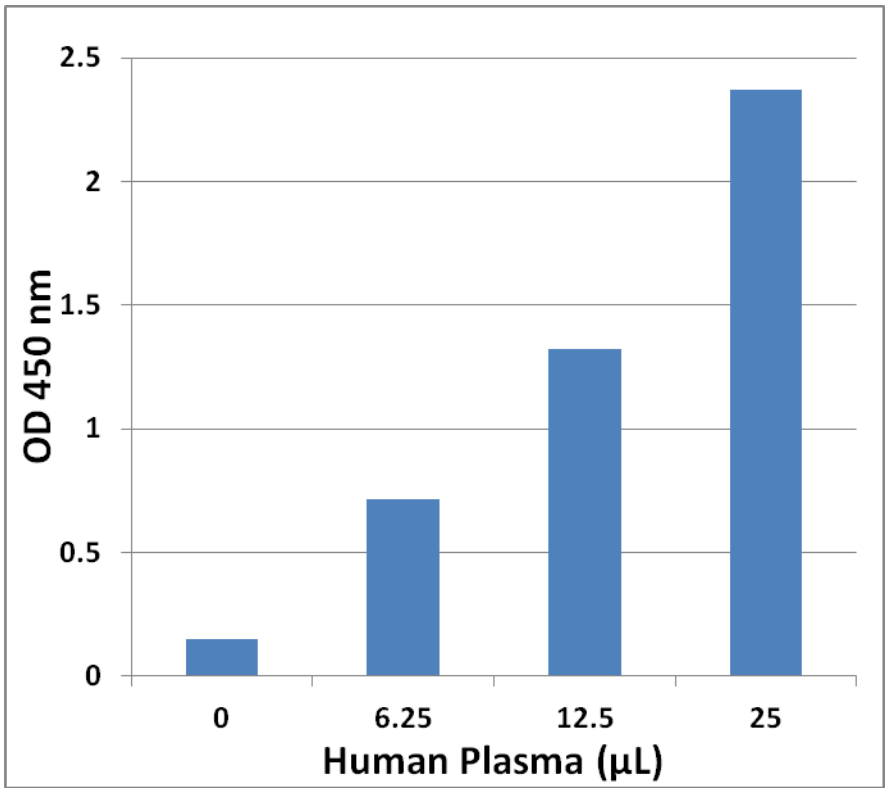

Figure 3: Detection of soluble LRP1 in human plasma.

References

1. Lillis A.P., Van Duyn L.B., Muphy-Ullrich J.E., and Strickland D.K. (2011). *Physiol. Rev.* **88**, 887-918.
2. Willnow TE, Armstrong SA, Hammer RE, and Herz J. (1995). *Proc Natl Acad Sci USA* **92**, 4537-4541.
3. Pietrzik C.U., Busse T., Merriam D. E., Weggen S., and Koo E.H.. (2002). *EMBO J* **21**, 5691-5700.
4. Rohlmann A., Gotthardt M., Hammer R.E., and Herz J. (1998) *J Clin Invest* **101**, 689-695.
5. Newton C.S., Loukinova E., Mikhailen I., Ranganathan S., Gao Y., Haudenschild C., and Strickland D.K. (2005) *J Biol Chem* **280**, 27872-27878.
6. Hofmann S.M., Zhou L., Perez-Tilve D., Greer T., Grant E., Wancata L., Thomas A., Pfluger P.T., Basford J.E., Gilham D., Herz J., Tschop M.H., and Hui D.Y. (2007) *J Clin Invest* **117**, 3271-3282.
7. Orr AW, Pallero M.A., and Murphy-Ullrich J.E. (2002) *J Biol Chem* **277**, 20453-20640.
8. Ogden C.A., deCathelineau A., Hoffman P.R., Bratton D., Ghebrehiwet B., Fadok V.A., and Henson P.M. (2001) *J Exp Med* **194**, 781-795.

Warranty

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