

HUMAN LRG1 ELISA

Life Diagnostics, Inc., Catalog Number: LRG-20

****FOR RESEARCH USE ONLY****

INTRODUCTION

Leucine-rich alpha-2-glycoprotein-1 (LRG1) is a 50 kDa serum protein that is primarily expressed in liver. It is a positive acute phase reactant; serum levels are elevated during chronic inflammatory diseases and infections.^{1,2} It is also reported that LRG1 is upregulated in patients with ovarian and hepatocellular cancer.^{3,4}

PRINCIPLE OF THE ASSAY

The assay uses affinity purified human LRG1 antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated human LRG1 antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in LRG1 molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If LRG1 is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of LRG1 is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- LRG1 antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- LRG1 stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- 10x Diluent; YD25-10, 25 ml
- TMB, TMB11-1, 11 ml
- Stop solution, SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10x stock with nine volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The human LRG1 stock is provided in lyophilized form. Reconstitute with distilled or deionized water as detailed on the vial label (*the reconstituted stock is stable at 2-8°C for one day but should be aliquoted and frozen at -20°C after if future use is intended*).
2. Label 7 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
3. In the tube labeled 100 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of LRG1 stock and mix. This provides the 100 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
5. Prepare a 50 ng/ml standard by diluting and mixing 250 µl of the 100 ng/ml standard with 250 µl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

We found LRG1 concentrations of 16.4±8.2 µg/ml in serum from healthy individuals (mean±SD, n=5). To obtain values within range of the standard curve we suggest that samples be diluted 1000-fold prior to testing. A 1000-fold dilution can be achieved as follows.

1. For each sample to be tested, dispense 90 µl and 495 µl of diluent into two microcentrifuge tubes.
2. Mix 10 µl of serum or plasma with 90 µl of diluent in the first tube to give a 10-fold dilution.
3. Mix 5 µl of the 10-fold diluted sample with 495 µl of diluent in the second tube to give a 1000-fold dilution.

Optimal dilutions must be determined by the end user.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 2-8°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).

5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μ l of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 μ l of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 μ l of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

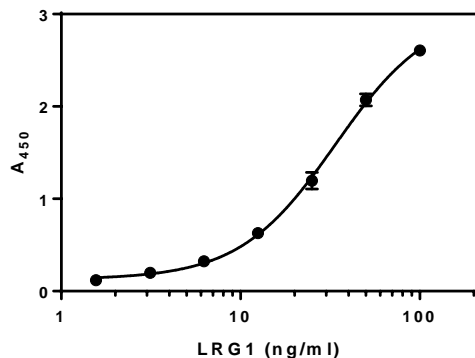
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus \log_{10} of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = \log_{10} concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with absorbance at 450 nm on the Y-axis against LRG1 concentrations on the X-axis is shown below. This curve is for illustration only.

LRG1 (ng/ml)	A_{450}
100	2.607
50	2.072
25	1.197
12.5	0.630
6.25	0.341
3.13	0.191
1.56	0.133



REFERENCES

1. Ha YJ. et. al. Usefulness of serum leucine-rich alpha-2-glycoprotein as a disease activity biomarker in patients with rheumatoid arthritis. *J Korean Med Sci.* 29:1199-1204 (2014)
2. Weivoda S. et. al. ELISA for human leucine-rich alpha-2-glycoprotein-1 employing cytochrome c as the capturing ligand. *J Immunol Methods.* 336:22-29 (2008)
3. Andersen JD. et. al. Leucine-rich alpha-2-glycoprotein-1 is upregulated in sera and tumors of ovarian cancer patients. *J. Ovarian Research.* 3:21 (2010)
4. Wang C-H. et. al. LRG1 expression indicates unfavorable clinical outcome in hepatocellular carcinoma. *Oncotarget.* 6(39):42118-42129 (2015)

Rev 121417

For technical assistance please email us at
techsupport@lifediagnosics.com