INTRODUCTION

LECT2 (leukocyte cell-derived chemotaxin 2) is a ~16 kDa protein that behaves as a positive acute phase protein in mammals and fish. It was originally identified as a chemotactic factor but also has hepatokine properties. In studies at Life Diagnostics, we found that serum levels of LECT2 increased during bacterial infections.

PRINCIPLE OF THE ASSAY

The assay uses polyclonal antibodies generated against Rainbow Trout LECT2. Horseradish Peroxidase (HRP) conjugated antibodies are used for detection. Standards and diluted samples (100 µl) are incubated in the antibody coated microtiter wells for 45 minutes. After washing the wells, HRP-conjugate (100 µl) is added and incubated for 45 minutes. If LECT2 molecules are present, they are sandwiched between the capture and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If LECT2 is present, a blue color develops. Color development is stopped after 20-minutes by addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of LECT2 is proportional to absorbance and is derived from a standard curve.

MATERIALS

Materials provided with the kit:

- Anti-LECT2 coated plate (12 x 8-well strips)
- HRP conjugate stock.
- LECT2 stock, 2 vials. **Store at -20°C**
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 2 x 50 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 tubes or 96-well polystyrene plates
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE

Store the standard stock vials at -20°C. The remainder of the kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. The kit 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 conducted with a complete understanding of the instructions and with adherence to good laboratory practice.
3. It is important that standards and samples be added to the ELISA plate quickly. If testing large numbers of samples, rather than pipetting standards and samples from individual tubes into the ELISA plate, we recommend the following: pipette an excess volume of standards and samples into wells of a blank polystyrene 96-well plate[1]. Then use an 8 or 12-channel multi-pipettor to quickly transfer 100 µl aliquots to the wells of the antibody-coated plate.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Laboratory temperature will influence absorbance readings. The assay was calibrated using a shaking incubator set at 150 rpm and 25°C. Performing the assay at lower temperatures and mixing speeds may result in lower absorbance values.

WASH SOLUTION

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. Unused wash buffer may be stored at 4°C for one week.

DILUENT

The diluent is formulated for measurement of LECT2 in trout and salmon serum or plasma. It is supplied ready to use. DO NOT substitute other buffers.

STANDARD

1. The stock is lyophilized. It is comprised of recombinant Rainbow Trout LECT2 in a stabilizing matrix. Reconstitute it with 200 µl of deionized water, gently mix, and prepare the 25 ng/ml standard as described on the vial label.
2. Label seven polypropylene tubes as 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0 ng/ml. Dispense 0.25 ml of diluent into each.
3. Pipette 0.25 ml of the 25 ng/ml LECT2 standard into the tube labeled 12.5 ng/ml and mix. This provides the 12.5 ng/ml LECT2 standard.
4. Similarly prepare the 6.25 – 0.39 ng/ml standards by two-fold serial dilution.

If future use is intended, the reconstituted standard stock should be stored frozen at or below -20°C within 5 minutes of reconstitution.

[1] Standards and sample dilutions may also be prepared directly in a blank polystyrene plate.

HRP CONJUGATE

The HRP conjugate stock must be diluted with diluent YD50-1 as described on the stock vial label about 5 minutes before use. Use 100 µl of the diluted HRP conjugate per well.

SAMPLES

We found LECT2 levels of approximately 1.5 µg/ml in serum from healthy trout. Levels increased to 10 µg/ml or higher three to six days after bacterial infection. To obtain values within range of the standard curve we found it necessary to dilute trout serum at least 800-fold. Do not test serum at dilutions less than 800-fold because matrix effects may occur. The diluent provided with this kit (YD50-1) must be used for dilution. Do not substitute other buffers.

PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Unused strips should be stored in a sealed bag with desiccant at 4°C.
2. Dispense 100 µl of standards and samples into appropriate wells. We recommend that standards and samples be tested in duplicate.
3. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
5. Dispense 100 µl of diluted HRP conjugate into the wells.
6. Incubate on a plate shaker at 150 rpm and 25°C for 45-minutes.
7. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 µl of TMB into each well.
10. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. After 20 minutes stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read absorbance at 450 nm[2] with a plate reader within 5 minutes.

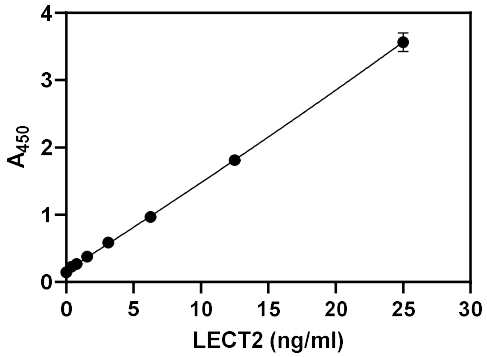
RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the LECT2 concentration. We suggest using a second order polynomial (quadratic) equation.
2. Derive the concentration of LECT2 in the samples.
3. Multiply the derived concentration by the dilution factor to determine the concentration in the sample.
4. If the absorbance values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only.

LECT2 (ng/ml)	A_{450}
25	3.567
12.5	1.811
6.25	0.968
3.13	0.589
1.56	0.378
0.78	0.268
0.39	0.228
0	0.144



[2] If absorbance of the high standard is ≥4 when measured at 450 nm, absorbance of all standards and samples should be read at 405 nm.

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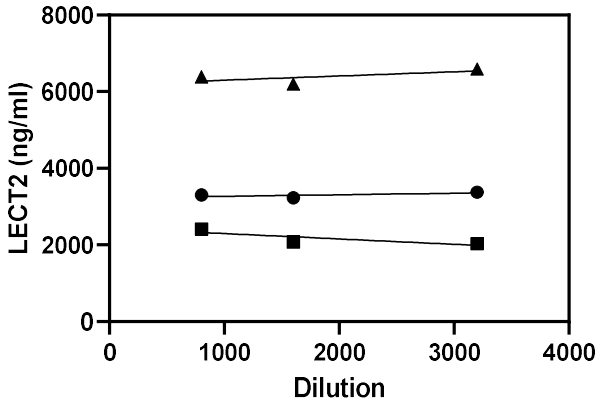
PERFORMANCE

Linearity: To assess the linearity of the assay, three Rainbow Trout plasma samples with LECT2 concentrations of 2270, 3320, and 6725 ng/ml were serially diluted to give values within range of the assay.



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For technical assistance please email us: techsupport@lifediagnostics.com

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