INTRODUCTION

Complement Factor H Like 1 (CFHL-1) serves a regulatory role in complement activation. It is expressed in the liver and circulates in blood. Levels are high in rainbow trout resistant to bacterial infections.

PRINCIPLE OF THE ASSAY

The assay uses polyclonal antibodies generated against recombinant Rainbow Trout CFHL-1. Unconjugated antibodies are coated on wells of a microtiter plate and used for capture. Horseradish Peroxidase (HRP) conjugated antibodies are used for detection. Standards and denatured/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 CFHL-1 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 CFHL-1 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 CFHL-1 is proportional to absorbance and is derived from a standard curve.

MATERIALS

Materials provided with the kit:

- Anti-CFHL-1 coated plate (12 x 8-well strips)
- HRP conjugate stock.
- CFHL-1 stock, 2 vials. **Store at -20°C**
- Denaturing buffer: VDB10-1, 10 ml
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: SB50-1, 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 CFHL-1 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 pure recombinant Rainbow Trout CFHL-1 in a stabilizing matrix. Reconstitute it with 200 µl of deionized water, gently mix, and prepare the 50 ng/ml standard as described on the vial label.
2. Label seven polypropylene tubes as 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 ng/ml. Dispense 0.25 ml of diluent into each.
3. Pipette 0.25 ml of the 50 ng/ml CFHL-1 standard into the tube labeled 25 ng/ml and mix. This provides the 25 ng/ml CFHL-1 standard.
4. Similarly prepare the 12.5 – 0.78 ng/ml standards by two-fold serial dilution.

The standards should be prepared and used within 10 minutes of reconstitution of the stock. If future use of the reconstituted stock is intended, it should be re-frozen at or below -20°C within 10 minutes of reconstitution.

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

SAMPLES

Trout serum or plasma samples must be denatured, then further diluted prior to testing. The procedure listed below worked well for samples tested at Life Diagnostics.

Denaturation

1. Mix 10 µl of serum or plasma with 70 µl of VDB10-1 denaturing buffer (this gives an 8-fold dilution).
2. Incubate for exactly 30 minutes at room temperature.

Further Dilution

1. After denaturation, mix 2.5 µl of the denatured sample with 247.5 µl of SB50-1 diluent (this gives an 800-fold dilution).
2. Should greater dilution of the sample be necessary, denature the sample for exactly 30 minutes, then dilute the denatured 8-fold diluted sample appropriately with diluent SB50-1.

We highly recommend that denaturation and further dilution be performed in polystyrene 96-well plates (not provided). This allows for optimum control of the denaturation time, and easy transfer of denatured/diluted samples to the antibody-coated plate using 8 or 12-channel multi-pipettors.

HRP CONJUGATE

The HRP conjugate stock must be diluted with diluent SB50-1 as described on the stock vial label about 5 minutes before use. Use 100 µl of the diluted HRP conjugate per well.

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 denatured/diluted samples into appropriate wells.
3. Incubate on a 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. 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 5x 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.

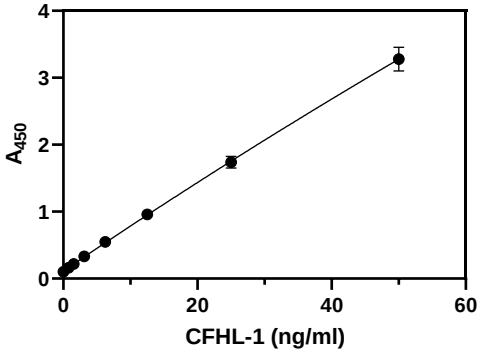
RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the CFHL-1 concentration. We suggest using a second order polynomial (quadratic) equation.
2. Derive the concentration of CFHL-1 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.

CFHL-1 (ng/ml)	A_{450}
50	3.279
25	1.740
12.5	0.958
6.25	0.548
3.13	0.330
1.56	0.217
0.78	0.163
0	0.103



[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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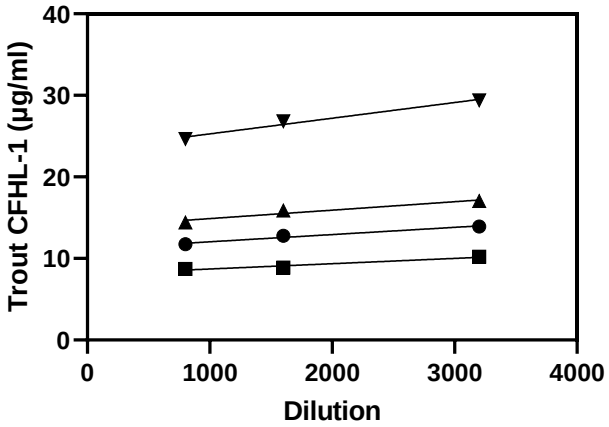
PERFORMANCE

Linearity: To assess the linearity of the assay, four rainbow trout plasma samples with CFHL-1 concentrations ranging from 9.3 to 29.8 μg/ml were serially diluted to produce values within the dynamic range of the assay.



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For technical assistance please email us at info@lifediagnostics.com

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