KAMIYA BIOMEDICAL COMPANY

Trout C1q-Like Protein 3 ELISA

For the quantitative determination of C1q-Like Protein 3 in trout serum or plasma

Cat. No. KT-1917

For Research Use Only.

K-ASSAY ®

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Trout C1q-Like Protein 3 (C1q-LP3) ELISA is an enzyme immunoassay for the quantitative determination of C1q-LP3 in trout serum or plasma. For research use only.

INTRODUCTION
C1q-like protein 3 (C1q-LP3) was identified in proteomic studies as a protein that is increased in rainbow trout and Atlantic salmon plasma during bacterial infections. Levels in healthy fish are ~ 0.1 µg/mL, increasing up to 70 µg/mL during infection.

PRINCIPLE
The assay uses a polyclonal antibody generated against recombinant rainbow trout C1Q-LP3. It recognizes rainbow trout and Atlantic salmon C1q-LP3. The unconjugated antibody is coated on wells of a microtiter plate and used for capture. A horseradish peroxidase (HRP) conjugate is used for detection. Calibrators 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 C1q-LP3 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 C1q-LP3 is present, a blue color develops. Color development is stopped by addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of C1q-LP3 is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
• Anti-C1q-LP3 coated plate (12 x 8-well strips)
• HRP conjugate, 11 mL
• C1q-LP3 stock calibrator, 1 vial, **Store at -20°C.**
• 20x Wash Solution, 50 mL
• Diluent, 2 x 50 mL
• TMB, 11 mL
• Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized 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

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 calibrators and samples be added to the ELISA plate quickly. If testing large numbers of samples, rather than pipetting calibrators and samples from individual tubes into the ELISA plate, we recommend the following. First, pipette an excess volume of calibrators and samples into wells of a blank polystyrene 96-well plate. Then use an 8 or 12-channel multi-pipettor to quickly transfer 100 µL aliquots to the appropriate 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.

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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 de-ionized water. Unused wash buffer may be stored at 4°C for one week.

DILUENT PREPARATION

The diluent is formulated for measurement of C1q-LP3 in trout and salmon serum or plasma. It is supplied ready to use. DO NOT substitute other buffers.

CALIBRATOR

1. The stock is lyophilized. Reconstitute it with the volume of diluent shown on the vial label and prepare the 100 ng/mL calibrator as described.
2. Label seven polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 ng/mL. Dispense 0.25 mL of diluent into each.
3. Pipette 0.25 mL of the 100 ng/mL C1q-LP3 calibrator into the tube labeled 50 ng/mL and mix. This provides the 50 ng/mL C1q-LP3 calibrator.
4. Similarly prepare the 25 – 1.56 ng/mL calibrators by two-fold serial dilution.
Unused reconstituted C1q-LP3 should be stored at or below -20°C.

SAMPLE PREPARATION

In studies, we found C1q-LP3 levels ranging from 0 to >70 µg/mL. Optimal dilutions must be determined empirically. We suggest that samples initially be evaluated at a dilution of 100-fold (2.5 µL of serum or plasma mixed with 247.5 µL of diluent). Ideally dilutions should be performed in polystyrene 96-well plates (not provided). This allows quick and easy transfer of diluted samples to the antibody-coated plate using 8- or 12-channel multi-pipettors.

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

CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus the C1q-LP3 concentration.
2. Fit the calibration curve using graphing software. We suggest using a second order polynomial (quadratic) equation.
3. Derive the concentration of C1q-LP3 in the samples.
4. Multiply the derived concentration by the dilution factor to determine the concentration in the sample.
5. If the absorbance values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

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

C1q-LP3 (ng/mL)	A_{450}
100	3.586
50	2.025
25	1.079
12.5	0.704
6.25	0.473
3.13	0.308
1.56	0.231
0	0.211
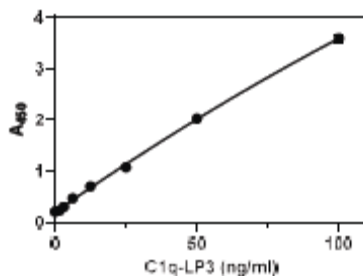
ASSAY PERFORMANCE

Linearity: To assess the linearity of the assay, four rainbow trout plasma samples with C1q-LP3 concentrations of 5.6, 21.9, 43.6, and 73.1 µg/mL were serially diluted to produce values within the dynamic range of the assay.
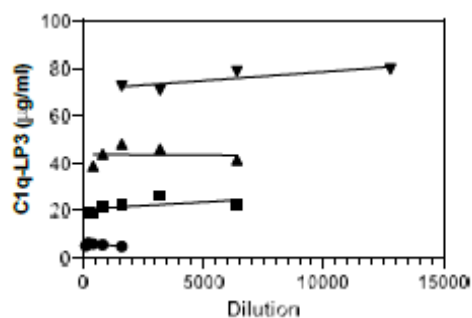
STORAGE

Store the calibrator vial 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 until the expiration date.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY

12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068   Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com