

KAMIYA BIOMEDICAL COMPANY

Trout Cathelicidin ELISA

For the quantitative determination of cathelicidin in trout serum or plasma

Cat. No. KT-1921

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Trout Cathelicidin ELISA is an enzyme immunoassay for the quantitative determination of cathelicidin in trout serum or plasma. For research use only.

INTRODUCTION

Cathelicidins are small antimicrobial proteins. Expression levels increase in Rainbow Trout and Atlantic Salmon during bacterial infections.

PRINCIPLE

The assay uses a polyclonal antibody that recognizes Rainbow Trout and Atlantic Salmon Cathelicidin-2 (referred to here as Cathelicidin). Unconjugated antibody is coated on wells of a microtiter plate and used for capture. Horseradish peroxidase (HRP) conjugated antibody 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 Cathelicidin 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 Cathelicidin 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 Cathelicidin is proportional to absorbance and is derived from a calibration curve.

COMPONENTS

- Anti-cathelicidin coated plate (12 x 8-well strips)
- 2x HRP conjugate, 7 mL
- Cathelicidin stock calibrator, 2 vials. **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: 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 μ L 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 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 cathelicidin in trout and salmon serum or plasma. It is supplied ready to use. DO NOT substitute other buffers.

CALIBRATOR PREPARATION

1. The stock is lyophilized. Reconstitute it with 200 μ L of de-ionized water, gently mix, and prepare the 40 ng/mL calibrator as described on the vial label.
 2. Label seven polypropylene tubes as 20, 10, 5, 2.5, 1.25, 0.625, and 0 ng/mL. Dispense 0.25 mL of diluent into each.
 3. Pipette 0.25 mL of the 40 ng/mL cathelicidin calibrator into the tube labeled 20 ng/mL and mix. This provides the 20 ng/mL cathelicidin calibrator.
 4. Similarly prepare the 10 – 0.625 ng/mL calibrators by two-fold serial dilution.
- Discard the stock after use.

HRP CONJUGATE PREPARATION

For each 8-well strip used in the assay, mix 0.5 mL of 2x HRP conjugate with 0.5 mL of diluent. Use 100 μ L per well.

SAMPLE PREPARATION

Trout. We found Cathelicidin levels ranging from approximately 1 μ g/mL in plasma from healthy fish to approximately 120 μ g/mL in plasma from fish infected with *Flavobacterium psychrophilum*. Because of the wide range, optimal dilutions must be determined empirically. However, we suggest that samples initially be tested at a 1,000-fold dilution.

Salmon. We found that Cathelicidin levels ranged from approximately 0.3 μ g/mL in plasma from healthy salmon to approximately 1.2 μ g/mL in plasma from salmon with pancreatic disease. We suggest testing salmon plasma at a 100-fold dilution.

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 μ L 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 μ L/well).
5. Dispense 100 μ L of 1x 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 μ L/well).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 μ L 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 μ L 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 with a plate reader within 5 minutes.

CALCULATION OF RESULTS

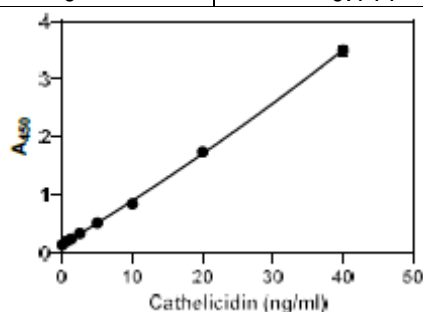
1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus the cathelicidin concentration.
2. Fit the calibration curve using graphing software. We suggest using a second order polynomial (quadratic) equation.

3. Derive the concentration of cathelicidin 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

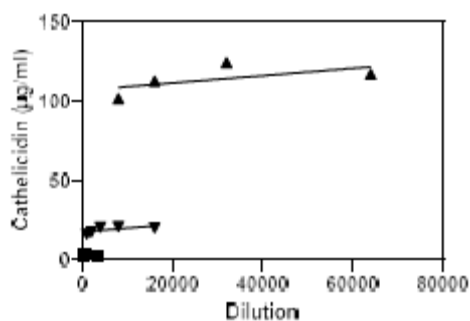
A typical calibration curve is shown below. This curve is for illustration only.

Cathelicidin (ng/mL)	A ₄₅₀
40	3.498
20	1.748
10	0.853
5	0.524
2.5	0.341
1.25	0.245
0.625	0.207
0	0.144



ASSAY PERFORMANCE

Linearity: To assess the linearity of the assay, three rainbow trout plasma samples with cathelicidin concentrations of 3.1, 19.1 and 113.6 $\mu\text{g/mL}$ 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

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