

**KAMIYA BIOMEDICAL COMPANY**

# Salmon Cardiac Troponin-C ELISA

**For the quantitative determination of Cardiac Troponin-C (CTNC) in salmon serum**

**Cat. No. KT-1911**

**For Research Use Only.**

## PRODUCT INFORMATION

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#### PRODUCT

The **K-ASSAY®** Salmon Cardiac Troponin-C ELISA is an enzyme immunoassay for the quantitative determination of Cardiac Troponin-C (CTNC) in salmon serum. For research use only.

#### INTRODUCTION

Troponin-C, a 17 kDa protein, is part of the troponin ITC-complex that regulates muscle contraction. It is expressed as two isoforms. One in fast twitch skeletal muscle (STNC), the other in cardiac and slow-twitch skeletal muscle (CTNC). This assay allows measurement of salmon and trout CTNC. Diseases that damage heart or slow-twitch skeletal muscle cause release of CTNC into serum. As shown in Figure 1, we found that serum CTNC levels were significantly increased in Atlantic salmon with pancreatic disease.

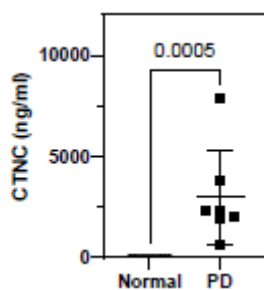


Figure 1. CTNC levels in serum from healthy salmon ( $3.0 \pm 1.5$  ng/mL, mean  $\pm$  SD,  $n = 13$ ) and salmon with pancreatic disease ( $3,378 \pm 2,342$  ng/mL, mean  $\pm$  SD,  $n=7$ ).

#### PRINCIPLE

The assay uses two different CTNC antibodies, one for solid phase immobilization and one, conjugated to horseradish peroxidase (HRP), for detection. Calibrators and diluted samples (100  $\mu$ L) are incubated in antibody coated microtiter wells for 45 minutes. After washing the wells, HRP-conjugate (100  $\mu$ L) is added and incubated for 45 minutes. If CTNC molecules are present, they are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If CTNC 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 CTNC is proportional to absorbance and is derived from a calibration curve.

#### COMPONENTS

- Anti-CTNC coated plate (12 x 8-well strips)
- HRP conjugate stock. **Store  $\leq -20^{\circ}\text{C}$ .**
- CTNC stock. **Store  $\leq -20^{\circ}\text{C}$ .**
- 20X Wash Solution, 50 mL
- Diluent, 2 x 50 mL. **Store  $\leq -20^{\circ}\text{C}$ .**
- TMB, 11 mL
- Stop Solution, 11 mL

#### MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettors and tips
- Distilled or de-ionized water
- Polypropylene tubes
- 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  $\mu$ L aliquots to the appropriate wells of the ELISA 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 specially formulated for measurement of CTNC in salmon serum. It is provided ready to use. DO NOT substitute other buffers. Thaw at room temperature, or in lukewarm water, and mix gently before use. Refreeze unused diluent at -20°C if future use is intended.

## CALIBRATOR

1. The stock is lyophilized. Reconstitute it with the volume of diluent indicated on the vial label and prepare the 10 ng/mL calibrator as instructed.
2. Label seven polypropylene tubes as 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 ng/mL. Dispense 0.25 mL of diluent into each.
3. Pipette 0.25 mL of the 10 ng/mL CTNC calibrator into the tube labeled 5 ng/mL and mix. This provides the 5 ng/mL CTNC calibrator.
4. Similarly prepare the 2.5 to 0.156 ng/mL calibrators by two-fold serial dilution. Unused reconstituted CTNC stock should be frozen at or below -20°C.

## HRP CONJUGATE

The HRP diluent is provided as a concentrated stock. Approximately five minutes prior to use dilute it with diluent as detailed on the stock vial label.

## SAMPLE PREPARATION

In studies, we found CTNC levels ranging from ~3 ng/mL in serum from healthy salmon to >7  $\mu$ g/mL in serum from salmon with pancreatic disease. To increase the possibility of obtaining values within range of the calibration curve we recommend that each sample be evaluated at dilutions of 100-fold and 2,000-fold. To avoid matrix effects do not use dilutions less than 50-fold.

## 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  $\mu$ 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  $\mu$ L/well).
5. Dispense 100  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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.

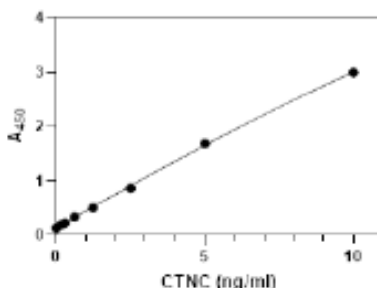
## CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus CTNC concentration.
2. Fit the calibration curve using graphing software. We typically fit to a two-site, total and nonspecific binding model, or a second order polynomial (quadratic) equation.
3. Multiply the derived concentration by the dilution factor to determine the concentration in the sample.
4. If the  $A_{450}$  values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

## TYPICAL CALIBRATION CURVE

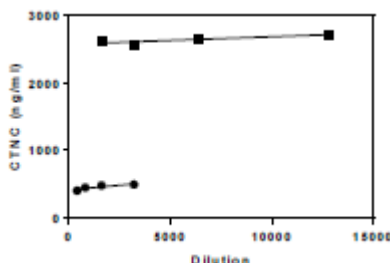
A typical calibration curve with absorbance at 450 nm on the Y-axis against CTNC concentrations on the X-axis is shown below. This curve is for illustration only.

CTNC (ng/mL)	A450
10	3.002
5	1.692
2.5	0.871
1.25	0.506
0.625	0.334
0.3125	0.226
0.156	0.194
0	0.136



## ASSAY PERFORMANCE

**Linearity:** To assess the linearity of the assay, two serum samples containing CTNC at concentrations of 443 and 2641 ng/mL were serially diluted to produce values within the dynamic range of the assay.



**Specificity:** The assay recognizes CTNC from mammals, salmon, and rainbow trout. Although not investigated. The antibodies used in the kit do not recognize fast-twitch skeletal muscle troponin-C from mammals or salmon.

## STORAGE

The CTNC stock, HRP conjugate stock, and diluent must be stored at  $-20^{\circ}\text{C}$ . The remainder of the kit should be stored at  $4^{\circ}\text{C}$  and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date.

## FOR RESEARCH USE ONLY

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