KAMIYA BIOMEDICAL COMPANY

# Trout HSP70 ELISA

**For the quantitative determination of HSP70 in trout tissue extracts**

## Cat. No. KT-1925

**For Research Use Only.**

# PRODUCT INFORMATION

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## PRODUCT

The **K-ASSAY®** Trout HSP70 ELISA is an enzyme immunoassay for the quantitative determination of HSP70 in trout tissue extracts. For research use only.

## INTRODUCTION

Heat Shock Protein 70 (HSP70) levels increase in Rainbow Trout heart, white muscle, red muscle, gill, and liver during heat stress. Studies have also shown that it is increased in Salmon gill, muscle, and liver in response to elevated water temperatures.

## PRINCIPLE

The assay uses a polyclonal antibody that recognizes Rainbow Trout and Salmon HSP70. Unconjugated antibodies are 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 one hour. After washing the wells, HRP-conjugate (100 µL) is added and incubated for 45 minutes. If HSP70 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 HSP70 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 HSP70 is proportional to absorbance and is derived from a calibration curve.

## COMPONENTS

- Anti-HSP70 coated plate (12 x 8-well strips)
- 2x HRP conjugate:. 7 mL
- HSP70 calibrator stock, 2 vials. Store at -20℃
- 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 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 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 HSP70 in trout and salmon tissue extracts. It is supplied ready to use. DO NOT substitute other buffers.

## CALIBRATOR PREPARATION

1. The stock is lyophilized. Reconstitute it with 200 µL of deionized water, gently mix, and prepare the 30 ng/mL calibrator as described on the vial label.
2. Label seven polypropylene tubes as 15, 7.5, 3.75, 1.88, 0.94, 0.47, and 0 ng/mL. Dispense 0.25 mL of diluent into each.
3. Pipette 0.25 mL of the 30 ng/mL HSP70 calibrator into the tube labeled 15 ng/mL and mix. This provides the 15 ng/mL HSP70 calibrator.
4. Similarly prepare the 7.5 – 0.47 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 µL per well.

## SAMPLE PREPARATION

We tested extracts from salmon muscle and rainbow trout spleen. Extracts were prepared by homogenizing tissue with four volumes of TBS (150 mM NaCl, 10 mM Tris.HCl, pH 7.4) using either a Potter Elvehjem homogenizer or a Bullet Blender®. Supernatants obtained after microcentrifugation were tested after being diluted a further 10-fold or greater with diluent. Because HSP70 levels vary with study conditions, optimal dilutions must be determined empirically.

## 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 one hour.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µL/well).
5. Dispense 100 µL 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 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 with a plate reader within 5 minutes. If absorbance of the high calibrator is ≥4 when measured at 450 nm, absorbance of all calibrators and samples should be read at 405 nm.

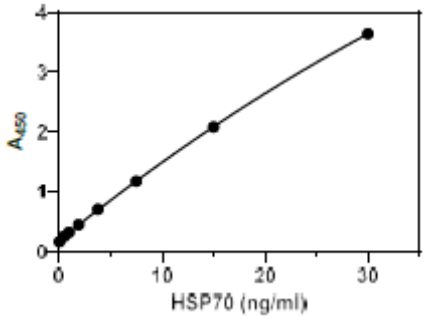
## CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus the HSP70 concentration. We suggest using a second order polynomial (quadratic) equation.
2. Derive the concentration of HSP70 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 calibration curve, samples should be diluted appropriately and re-tested.

## TYPICAL CALIBRATION CURVE

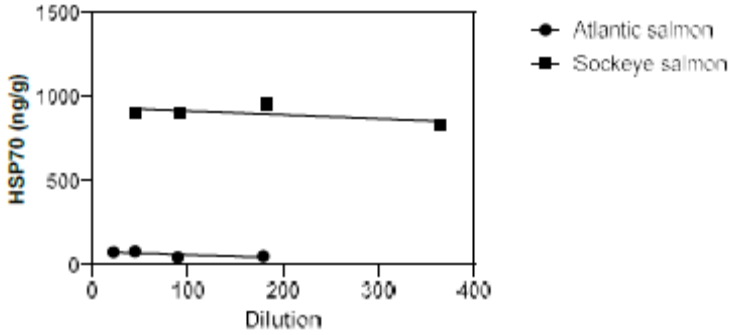
A typical calibration curve is shown below. This curve is for illustration only.

| HSP70 (ng/mL) | $A_{450}$ |
|---|---|
| 30 | 3.640 |
| 15 | 2.079 |
| 7.5 | 1.171 |
| 3.75 | 0.701 |
| 1.88 | 0.447 |
| 0.94 | 0.314 |
| 0.47 | 0.252 |
| 0 | 0.158 |




## ASSAY PERFORMANCE

**Linearity:** To assess the linearity of the assay, muscle extracts from healthy Atlantic and Sockeye salmon were serially diluted to produce values within the dynamic range of the assay.




## STORAGE

Store the calibrator vial at -20℃. The remainder of the kit should be stored at 4℃ 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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