K-ASSAY®

KAMIYA BIOMEDICAL COMPANY

Fish cortisol ELISA

For the quantitative determination of endogenic fish cortisol in serum, plasma and tissue homogenates

Cat. No. KU-143

For Research Use Only.

Rev. 15849143

Product Information
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INTENDED USE

The Fish cortisol ELISA is for the quantitative determination of endogenic fish cortisol in serum, plasma and tissue homogenates. For research use only.

PRINCIPLE

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antigen. Calibrators or samples are added to the appropriate microtiter plate wells with antibody specific for Cortisol and Horseradish Peroxidase (HRP) conjugated goat-anti-rabbit antibody. The competitive inhibition reaction is launched between pre-coated Cortisol and Cortisol in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of Cortisol in the sample. The color development is stopped, and the intensity of the color is measured.

PERFORMANCE

Detection Range

3.12 – 200.00 ng/mL.

Sensitivity

The minimum detectable dose of fish cortisol is typically less than 1.56 ng/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest fish cortisol concentration that could be differentiated from zero.

Specificity

This assay has high sensitivity and excellent specificity for detection of fish cortisol. No significant cross-reactivity or interference between fish cortisol and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between fish cortisol and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-assay Precision (Precision within an assay): CV% <8%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV% <10%

Three samples of known concentration were tested in twenty assays to assess.

Limitations of the Procedure

- **For research use only. Not for use in diagnostic procedures**.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate higher values than the highest calibrator, dilute the samples with Sample Diluent and repeat the assay.
- Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

COMPONENTS

	Quantity
Microtiter plate	1 (96 wells)
Plate sealer for 96 wells	4
Calibrator (Lyophilized)	2
HRP-Conjugate (100x concentrate)	1 x 120 µL
HRP-Conjugate Diluent	1 x 20 mL
Sample Diluent	2 x 20 mL
Wash Buffer (25x concentrate)	1 x 20 mL
TMB Substrate	1 x 10 mL
Stop Solution	1 x 10 mL
Antibody (100x concentrate)	1 x 60 µL
Antibody Diluent	1 x 10 mL

STORAGE

Unopened kit: Store at 4°C. Do not use the kit beyond the expiration date.

Opened kit: May be stored up to 1 month at 4°C. Try to keep the plate in a sealed aluminum foil bag and avoid moisture. If long-term use is intended for the Calibrator, Antibody and HRP-Conjugate it is advisable to store these reagents at -20°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. An incubator which can provide stable incubation conditions up to 37°C ± 0.5°C.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100 mL and 500 mL graduated cylinders.
6. De-ionized or distilled water.
7. Pipettes and pipette tips.
8. Test tubes for dilution.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates

100 mg tissue was rinsed with 1X PBS, homogenized in 1 mL of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5,000 x g, 4°C. The supernate was removed and assayed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Dilute the serum or plasma samples with **Sample Diluent** (1:100) before test. The suggested 100-fold dilution can be achieved by adding 10 µL sample to 40 µL of **Sample Diluent**. Complete the 100-fold dilution by adding 15 µL of this solution to 285 µL of **Sample Diluent**. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

Note:

1. Kamiya Biomedical Company is only responsible for the kit itself, not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 4°C. Otherwise, samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual for use in this assay.
5. Predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
7. Owing to the possibility of mismatching between antigens from other sources and the antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
8. Influenced by cell viability, cell number and sampling time, etc.; samples from cell culture supernatant may not be detected by the kit.
9. Fresh samples that have not been in storage long, are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

1. Use graduated containers to prepare the reagent. **Do not** prepare the reagent directly in the Diluent vials provided in the kit.
2. Bring all reagents to room temperature (18-25°C) 30 minutes before use.
3. Prepare fresh calibrator for each assay. Use within 4 hours and discard after use.
4. Making serial dilution directly within the wells is not permitted.
5. Carefully reconstitute Calibrators according to the instructions, avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.
6. Distilled water is recommended to make the preparation for reagents. Contaminated water or containers for reagent preparation will influence the detection result.

Antibody (1x)

Centrifuge the vial before opening. The Antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Antibody + 990 µL of **Antibody Diluent**.

HRP-Conjugate (1x)

Centrifuge the vial before opening. **HRP-Conjugate** requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of HRP-Conjugate + 990 µL of HRP-Conjugate Diluent.

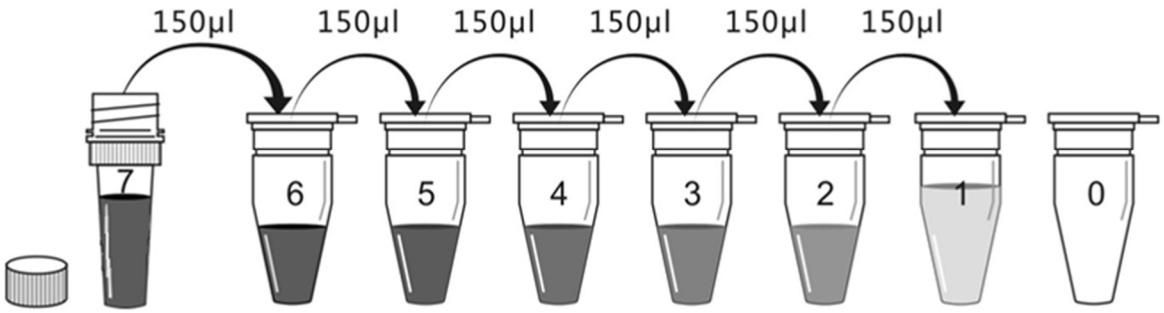
Wash Buffer (1x)

If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of **Wash Buffer Concentrate** (25x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1x).

Calibrator

Centrifuge the calibrator vial at 6,000 - 10,000 rpm for 30s before opening.
Reconstitute the **Calibrator** with 1.0 mL of **Sample Diluent**. Do not substitute other diluents. This reconstitution produces a stock solution of 200 ng/mL. Mix the calibrator to ensure complete reconstitution and allow the calibrator to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 150 µL of **Sample Diluent** into each tube (C0-C6). Use the stock solution to produce a **2-fold** dilution series (see below). Mix each tube thoroughly before the next transfer. The undiluted Calibrator serves as the high calibrator (200 ng/mL). **Sample Diluent** serves as the zero calibrator (0 ng/mL).



Tube	C7	C6	C5	C4	C3	C2	C1	C0
ng/ml	200	100	50	25	12.5	6.25	3.12	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and calibrators be assayed in duplicate.

1. Prepare all reagents, working calibrators, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc bag. store unused wells at 4°C.
3. Set a **Blank** well without any solution.
4. Add 50 µL of **calibrator** and **sample** per well. Add 50 µL **Antibody (1x)** to each well immediately (not to the Blank well). Mix well with the pipette or shake the plate gently for 60 seconds. A plate layout is provided to record standards and samples assayed.
5. Cover with the adhesive strip provided. Incubate for 40 minutes at 37°C.
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with **Wash Buffer** (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining **Wash Buffer** by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100 µL **HRP-conjugate (1x)** to each well immediately (not to Blank well). Cover with the adhesive strip provided. Incubate for 30 minutes at 37°C.
8. Repeat the aspiration/wash process five times as in step 6.
9. Add 90 µL of **TMB Substrate** to each well. Incubate for 20 minutes at 37°C. **Protect from light.**

10. Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set it to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

***Samples may require dilution. Please refer to the Sample Preparation section.**

Note:

1. The final experimental results will be closely related to operation skills of the end users and the experimental environments.
2. **Sample or reagents addition:** Use freshly prepared Calibrator. Carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all calibrators and samples, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each calibrator level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Incubation:** To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, Do not let the strips dry out at any time during the assay. Incubation time and temperature must be controlled.
4. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. **Controlling reaction time:** Observe the change of color after adding **TMB Substrate** (e.g. observation once every 10 minutes), if the color is too deep, add **Stop Solution** in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading. **TMB Substrate** should change from colorless or light blue to gradations of blue.
6. **TMB Substrate** is easily contaminated. The **TMB Substrate** should remain colorless or light blue until added to the plate. Protect it from light.
7. **Stop Solution** should be added to the plate in the same order as the **TMB Substrate**. The color developed in the wells will turn from blue to yellow upon addition of the **Stop Solution.** Wells that are green in color indicate that the **Stop Solution** has not mixed thoroughly with the **TMB Substrate.**

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples and calibrators as instructed.
2. Set a Blank well without any solution.
3. Add 50 µL calibrator or sample to each well.
4. Add 50 µL Antibody (1x) to each well (not to blank well).
5. Incubate for 40 minutes at 37°C.
6. Aspirate and wash 3 times.
7. Add 100 µL HRP-conjugate (1x) to each well (not to blank well).
8. Incubate for 30 minutes at 37°C.
9. Aspirate and wash 5 times.
10. Add 90 µL TMB Substrate to each well. Incubate 20 minutes at 37°C. Protect from light.
11. Add 50 µL Stop Solution. Read at 450 nm within 5 minutes.

***Samples may require dilution. Please refer to the Sample Preparation section.**

CALCULATION OF RESULTS

1. Using professional graphing software to make the calibration curve is recommended.
2. Average the duplicate readings for each calibrator and sample and subtract the average optical density of the blank.

3. Create a calibration curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct the calibration curve by plotting the mean absorbance for each calibrator on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the cortisol concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.
4. If samples have been diluted, the concentration read from the calibration curve must be multiplied by the dilution factor.

FOR RESEARCH USE ONLY.

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