Fish Luteinizing Hormone (LH) ELISA Kit

Catalog No: abx051240

Size: 96T

Range: 0.312 ng/ml - 5 ng/ml

Sensitivity: 0.1 ng/ml

Storage: Store at 2-8°C for 6 months.

Application: The quantitative detection of LH in Fish serum, plasma and other biological fluids.

Principle of the Assay: This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody is pre-coated onto a 96-well plate. Standards, test samples and HRP-conjugated reagent are added to the wells and incubated. Unbound conjugates are removed using wash buffer. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient LH will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the LH amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of LH can be calculated.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Fish FSH Standard: 0.3 ml x 6 Tubes
- Wash buffer (20X): 25 ml
- Sample diluent buffer: 6 ml
- Detection Reagent: 10 ml
- Stop solution: 6 ml
- TMB substrate A: 6 ml
- TMB substrate B: 6 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 mins at 1000 x g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.
- **Other Biological Fluids:** Centrifuge at approximately 1000 × g for 20 mins to remove precipitate. Analyse immediately or aliquot and store at -20°C or -80°C.

Notes:

- Samples must be diluted so that the expected concentration falls within the kit's range. **Analyse samples at a 5-fold (1/5) dilution (i.e. add 10 µl of sample to 40 µl of Sample Diluent Buffer).**
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occassion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

B. Reagent Preparation

Standard:

6 tubes of Standard are provided. Please refer to the table below for the concentrations. The unit of concentration is ng/ml.

Tube	0	1	2	3	4	5
Concentration	0 ng/ml	0.31 ng/ml	0.63 ng/ml	1.25 ng/ml	2.5 ng/ml	5 ng/ml

Wash Buffer: Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 20 ml of concentrated wash buffer into 380 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

C. Assay Protocol

Prepare all standards, samples and reagents as directed above. Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate, and to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 50 µl of the diluted standards (5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.63 ng/ml, 0.31 ng/ml) into the standard wells.
3. Aliquot 50 µl of 0 ng/ml into the control (zero) well.
4. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
5. Cover the plate with a plate sealer and incubate for 30 mins at 37°C.
6. Remove the cover and discard the liquid. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.

7. Aliquot 100 µl of Detection Reagent A working solution to each well. Cover the plate with a plate sealer and incubate for 1 hr at 37°C.
8. Remove the cover and discard the solution. Wash the plate 5 times as explained in step 6.
9. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
10. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

For calculation, average the OD 450 readings for each reference standard, and each sample, and then subtract the average control (zero) OD reading.

(Relative OD) = (OD of Each Well) – (OD of Zero Well)

The standard curve can be plotted as the relative OD of each reference standard solution (X), against the respective concentration of each standard solution (Y). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the concentrations from interpolation by the dilution factor, to obtain the concentration before dilution.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of LH were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of LH were tested on 3 different plates, 8 replicates in each plate.

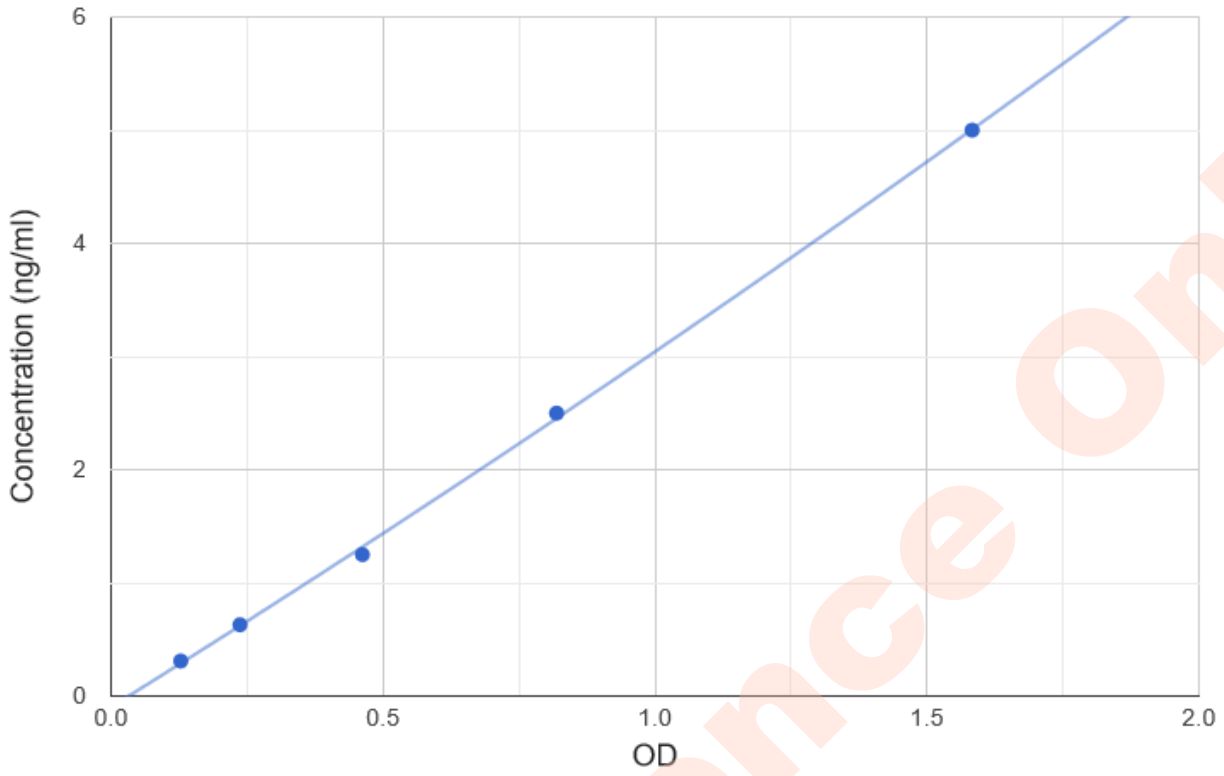
CV (%) = (Standard Deviation / Mean) × 100

Intra-Assay: CV < 10%

Inter-Assay: CV < 12%

D. Typical Data and Standard Curve

Typical Standard Curve Data is provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



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