GnRH ELISA Kit

09/21

(Catalog # E5099-100, 96 assays, Store at 4°C)

I. Introduction:

Gonadotropin-releasing hormone (GnRH) is produced and secreted by specialized nerve cells in the hypothalamus of the brain. It is released into tiny blood vessels that carry this hormone from the brain to the pituitary gland. GnRH is a releasing hormone responsible for the release of follicle-stimulating hormone and luteinizing hormone from the anterior pituitary. These hormones are released into the general circulation and act on the testes and ovaries to initiate and maintain their reproductive functions. Treatment with gonadotropin-releasing hormone agonists of uterine myoma, endometriosis and some hormone-dependent cancers, such as breast, ovarian, endometrial and prostate cancer, also seems to have a beneficial effect. **BioVision's GnRH ELISA Kit** is used to quantitatively measure GnRH in Serum, plasma and other biological fluids. The kit is based on the Competitive ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with GnRH. During the reaction, GnRH in samples or Standard competes with a fixed amount of GnRH on the solid phase supporter for sites on the Biotinylated Detection Ab specific to GnRH. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of GnRH in the samples is calculated by comparing the OD of the samples to the standard curve.

II. Features and Benefits:

- This ELISA kit is used for *in vitro* quantitative determination of GnRH concentrations in serum, plasma and other biological fluids.
- Detection Range: 15.63-1000 pg/ml
- Sensitivity: 9.38 pg/ml
- This competitive ELISA is highly sensitive and specific for the detection of GnRH. There is no significant cross-reactivity or interference between GnRH and analogues.

III. Sample Types:

Serum, plasma and other biological fluids

IV. Kit Contents:

Components	E5099-100	Part No.	Storage Temp
Micro ELISA Plate	8 X 12 Strips	E5099-100-1	-20°C
Standard	2 vials	E5099-100-2	-20°C
Biotinylated Detection Ab (100x)	120 µl	E5099-100-3	-20°C
HRP Conjugate (100x)	120 µl	E5099-100-4	-20°C
Standard & Sample Diluent	20 ml	E5099-100-5	4°C
Biotinylated Detection Antibody Diluent	14 ml	E5099-100-6	4°C
HRP Conjugate Diluent	14 ml	E5099-100-7	4°C
Wash Buffer (25x)	30 ml	E5099-100-8	4°C
Substrate Reagent	10 ml	E5099-100-9	4°C
Stop Solution	10 ml	E5099-100-10	4°C
Plate Sealer	5	E5099-100-11	RT

V. User Supplied Reagents and Equipment:

- Chemicals: Deionized or distilled water
- Microplate reader capable of measuring absorbance at 450 nm
- Absorbent paper

VI. Storage and Handling:

The entire kit can be shipped at 4 °C. If the kit is not supposed to be used within 1 month, store the items separately according to the above-mentioned conditions.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. HRP Conjugate: Calculate the required amount before the experiment (100 µl/well). Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent (Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).

2. Standard preparation: Centrifuge the standard at 10,000xg for 1 min. Add 1.0 mL of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 1000 pg/ml. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 1000, 500, 250, 125, 62.5, 31.25, 15.63, 0 pg/ml. Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube. Pipette 500 µl of the 1000 pg/ml

stock solution to the first tube and mix up to produce a 500 pg/ml working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay

3.Biotinylated Detection Antibody: Calculate the required amount before the experiment (50µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent(Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1:99)

4.Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved

5. Sample Preparation:

Note: Samples should be assayed within 7 days when stored at 4°C, otherwise aliquot and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles

• **Serum:** Allow samples to clot for 1 hour at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Tubes for blood collection should be disposable and be endotoxin free.

• **Plasma:** Collect plasma using EDTA-Na_2 as anticoagulant. Centrifuge samples for 15 min at 1000× g at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolyzed samples are not suitable for ELISA assay!

• **Cell lysates:** For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 µl of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10 min at 1500xg at 4°C. Remove the cell fragments, collect the supernatant for assay. Avoid repeated freeze-thaw cycles.

• **Tissue homogenates:** It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolyzed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant.

• **Cell culture supernatant or other biological fluids:** Centrifuge samples for 20 min at 1000×g at 2~ 8℃. Collect the supernatant for assay.

VIII. Assay Protocol:

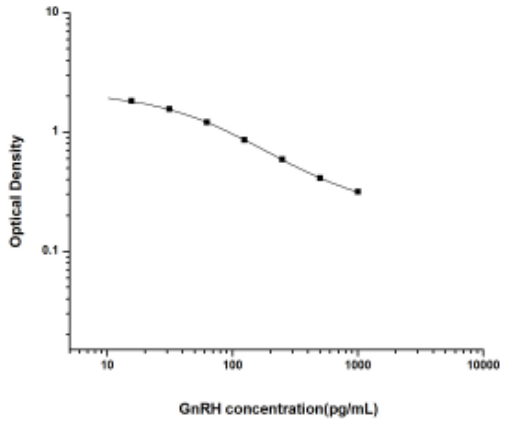
Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 50 µl of the **sample or standards** to separate duplicate wells.
2. Immediately add 50 µl diluted **Biotinylated Detection Antibody** to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C. Note: solutions should be added to the bottom of the micro-ELISA plate well, avoid touching the inside wall and bubble formation.
3. Aspirate the solution from each well, add 350 µl of 1x **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
4. Add 100 µl of diluted **HRP Conjugate** to each well. Cover with the **Plate sealer**. Incubate for 30 min at 37°C.
5. Aspirate the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90 µl of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 mins.
7. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Read the absorbance in micro plate reader set to 450 nm.

IX. Calculation:

Determine the average of the duplicate readings for each standard and samples. Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is under the lowest limit of the standard curve, retest the samples with appropriate dilution. The actual concentration is the concentration obtained by calculated multiplied by the dilution factor. Typical standard curve and data is provided below for reference only.

Conc. (pg/ml)	1000	500	250	125	62.5	31.25	15.63	0
OD	0.315	0.412	0.587	0.86	1.209	1.556	1.825	2.189



X. Recovery:

The recovery of GnRH spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices

Matrix	Recovery Range (%)	Average (%)
Serum (n=8)	93-111	101
EDTA Plasma (n=8)	88-100	95
Heparin Plasma (n=8)	87-97	92

XI. Linearity

Samples were spiked with high concentrations of GnRH and diluted with Standard and sample diluent to produce samples with values within the range of the assay.

Sample	1:2	1:4	1:8
Serum (n=5)	86-100%	93-106%	93-108%
EDTA Plasma (n=5)	92-100%	92-109%	90-101%
Cell culture media (n=5)	90-105%	91-102%	90-105%

XII. Related Products:

Thrombopoietin (Human) ELISA Kit (Cat. No. E4721)
Prostaglandin E2 (PGE2) ELISA Kit (Cat. No. E4637)
Oxytocin ELISA Kit (Cat. No. E4348)
Dihydrotestosterone (DHT) ELISA Kit (Cat. No. E4604)
Human Thyroid Stimulating Hormone (TSH) (Cat. No. P1031)

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