Follicle-Stimulating Hormone (human) ELISA Kit

Item No. 500710

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
400710	FSH Standard 1 (0 mIU/ml)	1 vial/1 ml
400711	FSH Standard 2 (5 mIU/ml)	1 vial/1 ml
400712	FSH Standard 3 (10 mIU/ml)	1 vial/1 ml
400713	FSH Standard 4 (25 mIU/ml)	1 vial/1 ml
400714	FSH Standard 5 (50 mIU/ml)	1 vial/1 ml
400715	FSH Standard 6 (100 mIU/ml)	1 vial/1 ml
400716	Streptavidin Precoated Plate	1 plate
400717	Anti-FSH-HRP + Anti-FSH-Biotin Conjugate	1 vial/12 ml
400718	TMB Substrate Solution	1 vial/15 ml
400719	Stop Solution	1 vial/15 ml
400827	Wash Solution (10X)	1 vial/50 ml
400828	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's Follicle-Stimulating Hormone (human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3641
Email:	techserv@caymanchem.com
Hours:	M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeating pipettor.
3. Materials used for **Sample Preparation** (see page 10).

INTRODUCTION

Background

Follicle-Stimulating hormone (FSH) is a glycoprotein produced by the pituitary gland and consists of two subunits with an approximate total molecular mass of 35 kDa. The α-subunit is identical to other pituitary hormones such as luteinizing hormone (LH), thyroid-stimulating hormone (TSH) and chorionic gonadotropin (hCG). The β-subunit is unique to FSH and confers the specific biological activity to the molecule. FSH secretion is stimulated by gonadotropin-releasing hormone (GnRH).

In males, FSH acts on the Sertoli cells of the testis to stimulate the synthesis of androgen-binding protein, which is indirectly involved in stimulating spermatogenesis. FSH also stimulates the secretion of inhibin, which acts by a negative feedback mechanism to inhibit further production of FSH by the pituitary. In females, FSH acts on the granulosa cells of the ovary to stimulate steroidogenesis, and to stimulate and sustain the development of a follicle leading up to ovulation.

About This Assay

Cayman's FSH (human) ELISA Kit is an immunometric (*i.e.*, sandwich) ELISA that permits FSH measurements within the range of 0.6-100 mIU/ml. This assay offers specific and sensitive analysis of FSH in human serum and has not been validated for other types of samples.

Principle of the Assay

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with streptavidin. Samples or standards, biotinylated capture antibody and an HRP-labeled detection antibody (Anti-FSH-HRP) are incubated in the wells. The biotinylated-capture antibody will bind both the streptavidin on the plate and any FSH introduced into the well, whereas the detection antibody will bind a different epitope on the FSH molecule. The entire complex is immobilized onto the wells by the streptavidin-biotinylated antibody interaction. After washing away excess, unbound reagents, the concentration of FSH is determined by measuring the enzymatic activity of HRP by adding the substrate tetramethylbenzidine (TMB). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color is directly proportional to the amount of bound anti-FSH-HRP, which in turn is proportional to the amount of FSH.

$$\text{Absorbance} \propto [\text{Anti-FSH HRP}] \propto [\text{FSH}]$$

A schematic of this process is shown in Figure 1, on page 8.

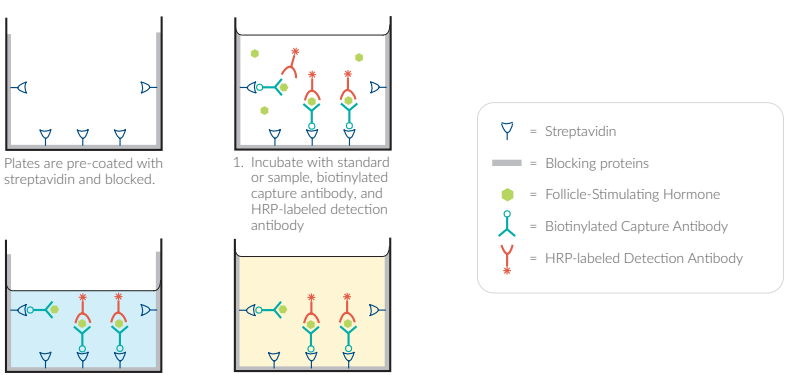


Figure 1. Schematic of the ELISA

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C.

Wash Buffer Preparation

50 ml vial Wash Solution (10X; Item No. 400827): Dilute to a total volume of 500 ml with distilled or deionized water.

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:10.

Sample Preparation

Human serum can be used directly in the assay.

To prepare serum samples for use in the ELISA, collect blood by venipuncture into tubes without additives or anti-coagulants and allow the blood to clot. Centrifuge the clotted blood at 1,000-2,000 x g for 15 minutes and carefully transfer the samples to clean tubes avoiding any lipid or cell debris in the tubes. For accurate comparison to established normal values, the serum sample should be collected after overnight fasting.

NOTE: Do not use heavily hemolyzed or highly lipemic samples. Store samples refrigerated (2°C-8°C) for a maximum of five days. If samples cannot be assayed within this time, store them at -20°C for up to 30 days. Avoid repetitive freeze-thaw cycles.

When assayed in dulpicate, 100 µl is required. If the concentration of FSH in the sample is greater than 100 mIU/ml, dilute an aliquot of the sample 1:1 with Standard 1 (0 mIU/ml).

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

NOTE: It is very important to bring all reagents, samples, and standards to room temperature (22-28°C) before starting the assay.

FSH Standard (Item Nos. 400710-400715)

Each of the six vials contains 1 ml standard solution at concentrations listed in the **Materials Supplied** section (see page 3), as well as listed on each vial. The standards are ready to use. After opening, the standard solutions are stable for six months if stored at 4°C.

Anti-FSH-HRP + Anti-FSH-Biotin Conjugate (Item No. 400717)

This vial contains 12 ml of a ready-to-use mixture of HRP-labelled Anti-FSH and biotin-labelled Anti-FSH antibodies.

TMB Substrate Solution (Item No. 400718)

This vial contains 15 ml of a ready-to-use TMB/hydrogen peroxide substrate solution. When stored in the dark at 4°C, the solution is stable for up to six months after opening. The solution should be colorless or have a slight blue tinge. If it is blue, it may have become contaminated and should not be used.

Stop Solution (Item No. 400719)

This vial contains 15 ml of 0.15 M sulfuric acid and is ready to use as supplied.

Plate Set Up

The 96-well plate included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all of the strips at once, plate the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk) and a six point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 2, see below. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest you record the contents of each well on the template sheet provided (see page 22).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	2	2	10	10	18	18	26	26	34	34
B	S2	S2	3	3	11	11	19	19	27	27	35	35
C	S3	S3	4	4	12	12	20	20	28	28	36	36
D	S4	S4	5	5	13	13	21	21	29	29	37	37
E	S5	S5	6	6	14	14	22	22	30	30	38	38
F	S6	S6	7	7	15	15	23	23	31	31	39	39
G	Blk	Blk	8	8	16	16	24	24	32	32	40	40
H	1	1	9	9	17	17	25	25	33	33	41	41

Blk - Blank
S1-S6 - Standards 1-6
1-41 - Samples

Figure 2. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

NOTE: Perform all assay steps in the order given and without appreciable delays between steps.Pipetting samples should not extend beyond ten minutes to avoid assay drift. TMB Substrate Solution and Stop Solution should be added in the same sequence.

Addition of the Reagents

1. **FSH Standards**

   Add 50 µl of each standard to appropriate wells.

2. **Samples**

   Add 50 µl of sample to appropriate wells.

3. **Anti-FSH-HRP + Anti-FSH-Biotin Conjugate**

   Add 100 µl of antibody mixture to each well, except the Blk wells.

Incubation of the Plate

Cover the plate with the plastic film and incubate at room temperature (22°C-28°C) for one hour.

Development of the Plate

1. Empty the wells and wash three times with diluted Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of TMB Substrate Solution to each well of the plate, including the Blk wells.
3. **Incubate for exactly 15 minutes** at room temperature in the dark.
4. DO NOT WASH THE PLATE OR EMPTY THE WELLS. Add 100 µl of Stop Solution to all wells and in the same order and same rate as the addition of TMB Substrate in Step 2.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.
3. The optical density (O.D.) of standard 6 should be ≥1.3.

ANALYSIS

Calculations

Standard Curve & Determination of Sample Concentration

Average the absorbance values of the Blk wells and subtract this value from the absorbance readings of each standard and sample well.

Using computer data reduction software, plot O.D. *versus* concentration for standards (S1-S6) and fit the data with a 4-parameter logistic, or alternatively, a smoothed cubic spline. Interpolate the concentration of your samples from the standard curve and be sure to correct for any dilution of the sample prior to addition to the well of the plate.

Reference Values

The following are expected ranges of FSH in human serum:

Males:		
	Before puberty	0-5.0 mIU/ml
	During puberty	0.3-10.0 mIU/ml
	Adult	1.5-12.4 mIU/ml
Females:		
	Follicular Phase	2.9-7.0 mIU/ml
	Lutheal Phase	1.2-8.8 mIU/ml
	Menopause	35-151 mIU/ml

Performance Characteristics

Sensitivity

The minimal detectable concentration of FSH by this assay is estimated to be 0.6 mIU/ml.

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

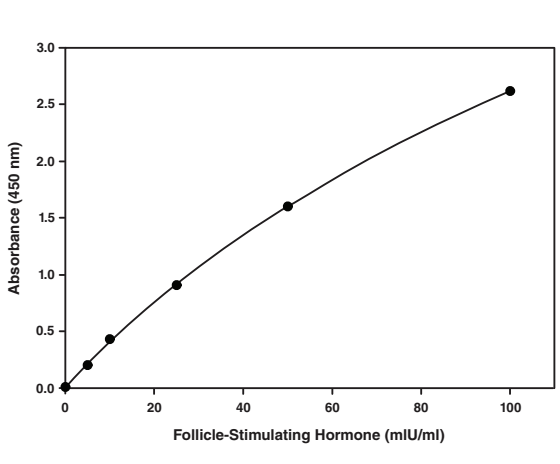


Figure 3. Typical standard curve

Precision

1. **Intra-assay variation**

   The precision within an assay was determined by 20 replicate determinations of three different control sera in the same assay.

Serum Sample	1	2	3
Number of Replicates	20	20	20
Mean FSH (mIU/ml)	5.4	15.6	37.9
Standard Deviation	0.46	1.32	3.56
Coefficient of Variation (%)	8.6	8.5	9.4

Table 1. Intra-Assay Variation

2. **Inter-assay variation**

   The precision between assays was determined by replicate measurements of three different control sera in several different assays.

Serum Sample	1	2	3
Number of Replicates	10	20	20
Mean FSH (mIU/ml)	5.9	16.9	35.3
Standard Deviation	0.66	1.59	4.16
Coefficient of Variation (%)	11.2	9.4	11.8

Table 2. Inter-Assay Variation

Cross Reactivity:

The cross reactivity of this kit to selected substances was evaluated by adding the potentially cross reacting substance to a serum matrix at various concentrations. The cross reactivity was calculated by deriving a ratio between the dose of test compound to dose of FSH needed to produce the same absorbance.

Substance	Cross Reactivity Ratio	Concentration
Follitropin (FSH)	1.0000	-
Lutropin (LH)	<0.0001	1,000 ng/ml
Chorionic gonadotropin (CG)	<0.0001	1,000 ng/ml
Thyrotropin (TSH)	<0.0001	1,000 ng/ml

Table 3. Cross Reactivity of the FSH Assay

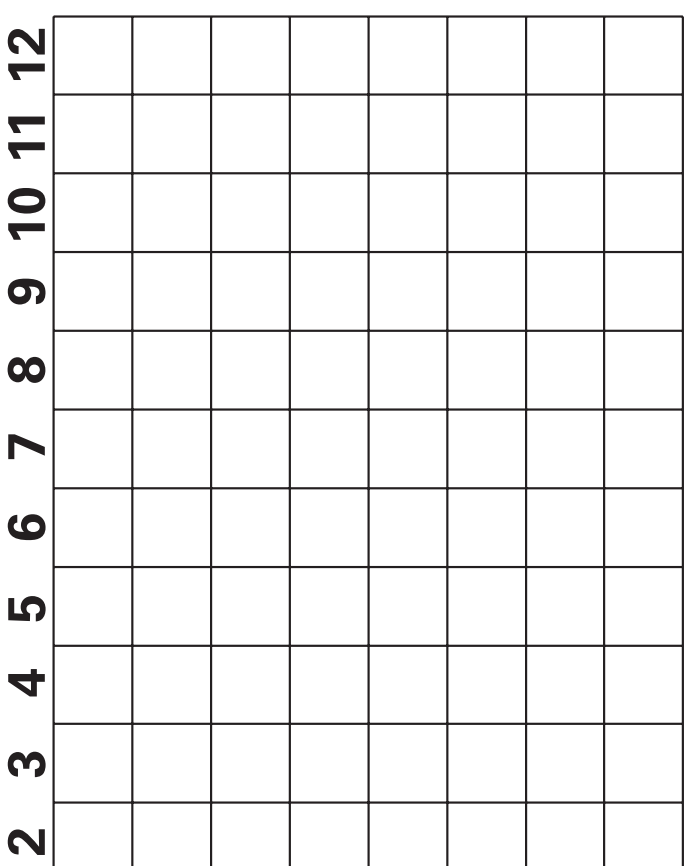
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal	A. Omission of key reagent B. Washes too stringent C. Incubation times inadequate D. Plate reader settings not optimal E. Incorrect assay temperature	A. Check that all reagents have been added in the correct order B. Use an automated plate washer if possible C. Use recommended incubation times D. Verify the wavelength and/or filter settings in the plate reader E. Use recommended incubation temperature; bring substrates to room temperature before use
High background	Inadequate washing	Ensure all wells are filled with Wash Buffer and are aspirated completely
Poor standard curve	A. Wells not completely aspirated B. Reagents poorly mixed C. Technique problem	A. Completely aspirate wells between steps B. Be sure that reagents are thoroughly mixed C. Proper mixing of reagents and wash steps are critical

Literature

1. Odell, W.D., Parlow, A.F., Cargille, C.M., *et al.* Radioimmunoassay for human follicle-stimulating hormone: Physiological studies. *J. Clin. Invest.* **47(12)**, 2551-2562 (1968).
2. Simoni, M., Gromoll, J., and Nieschlag, E. The follicle-stimulating hormone receptor: Biochemistry, molecular biology, physiology, and pathophysiology. *Endocr. Rev.* **18(6)**, 739-773 (1997).
3. Wennink, J.B., Delemarre-Van De Waal, H.A., Schoemaker, R., *et al.* Luteinizing hormone and follicle stimulating hormone secretion patterns in girls throughout puberty measured using highly sensitive immunoradiometric assays. *Clin. Endocrinol.* **33**, 333-344 (1990).
4. Layman, L.C., Lee, E.J., Peak, D.B., *et al.* Delayed puberty and hypogonadiam caused by mutations in the follicle-stimulating hormone β-subunit gene. *N. Engl. J. Med.* **337(9)**, 607-611 (1997).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.