

hFSH [I-125] IRMA KIT

(REF: RK-790CT)

The ¹²⁵I-hFSH IRMA system provides a direct quantitative *in vitro* determination of human Follicle Stimulating Hormone (hFSH) in human serum. hFSH can be assayed in the range of 0-150 mIU/ml using 100 µl serum samples.

Introduction

The Follicle Stimulating Hormone (Follicle Stimulating Hormone or hFSH) is a glycoprotein with a molecular weight of 30000, secreted by the adenohypophysis. Like other glycoprotein hormones (LH, TSH and HCG), hFSH contains two different subunits, an α-and a β-chain, linked by noncovalent bonds. The primary structures of the α subunits of hFSH and of those mentioned are virtually identical, whilst their β subunits are different. The β subunits are responsible for the immunological and biological specificity of these hormones.

The hFSH synthesis and release is stimulated by the hypothalamic Gonadotrophin-Releasing Hormone (GnRH), whereas the ovarian steroids secreted from the corpus luteum control further secretions of hFSH by negative feed-back.

The measurement of hFSH concentrations is an important part of the investigation of disorders of the hypothalamic-pituitary-gonadal axis. It is recommended to measure both hFSH and hLH to discriminate between hypothalamic and pituitary dysfunction.

Principle of the method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system. The ¹²⁵I labelled signal-antibody binds to an epitope of the FSH molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During a 1-hour incubation period with shaking immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter. The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hFSH, the unknown concentration of hFSH in patient samples can be determined.

Contents of the kit

1. 1 bottle of TRACER (21 ml), ready to use, containing about 740 kBq ¹²⁵I-anti-hFSH and capture anti-hFSH in buffer with red dye 0.1 % NaN₃.
2. 6 vials of STANDARDS (6 x 1.0 ml), (S1-S6). The exact concentrations are indicated

on each vial. (Calibrated against WHO 92/510 Int.Std.) in bovine serum with 0.1% NaN₃. See *Preparation of reagents*

3. 1 vial of CONTROL SERUM. Lyophilized human serum with 0.1% NaN₃. The concentration of the control serum is specified in the quality certificate enclosed. See *Preparation of reagents*.

4. 2 boxes of COATED TUBES, Ready to use. 2 x 50 reactive test tubes, 12x75 mm, packed in plastic boxes.

5. 1 bottle of WASH BUFFER CONCENTRATE (20 ml), containing 0.2% NaN₃. See *Preparation of reagents*.

Quality certificate
Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100, 200 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter

Recommended tools and equipment
repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-ml pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Samples with a hFSH concentration higher than that of the most concentrated standard should be diluted and reassayed.

Preparation of reagents, storage

Add the wash buffer concentrate (20 ml) to 700 ml distilled water to obtain 720 ml wash solution. Upon dilution store at 2-8°C until expiry date of the KIT.

Add 1000 µl distilled water to the lyophilized control serum. Mix gently with shaking or vortexing (foaming should be avoided).

Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes. Store at -20°C until the expiry date of the KIT.

Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date of the KIT. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Equilibrate reagents and samples to room temperature before use.
2. Label coated tubes in duplicate each standard (S1-S6), control serum and samples.

3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 100 µl of standards, control and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
5. Pipette 200 µl of tracer into each tube.
6. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm recommended).
7. Incubate tubes for 1 hour at room temperature.
8. Add 2.0 ml of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
9. Return the tube-rack to an upright position, and repeat step-8 one more time.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the hFSH concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		100		
Control			100	
Sample				100
Tracer	200	200	200	200
Shake for 1 hour at room temperature				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes.

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{2-6} / C / M_x (\text{cpm}) - S_1 (\text{cpm})}{T(\text{cpm})} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of hFSH.

Determine the hFSH concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Table 2. Typical assay data

Tubes	Mean cpm	B/T%
T	308164	-
S1	59	0.02
S2	364	0.12
S3	1810	0.59
S4	8414	2.73
S5	34174	11.1
S6	107452	34.9
C	7652	2.48

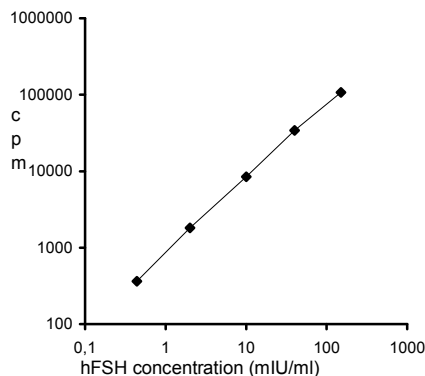


Figure 1: A typical standard curve
(Do not use to calculate unknown samples)

Characterization of the assay

Typical assay parameters

NSB/T < 0.1 %

Sensitivity

A detection limit of 0.08 mIU/ml has been obtained by assaying 20 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean cpm and its double standard deviation.

Specificity

No cross reactivity with hLH, hTSH and hCG can be detected in normal physiological concentrations.

Precision and Reproducibility

Five serum pools were assayed in 15 replicates to determine intra-assay precision. To determine inter-assay precision 5 serum pools were measured in duplicates in 15 independent assays by 2 operators using different kit batches. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean value (mIU/mL)	CV %	Mean value (mIU/mL)	CV %
0.24	6.1	0.26	7.6
1.61	2.6	1.68	3.7
6.5	2.5	6.7	2.7
26.0	0.7	26.2	1.3
84.6	3.0	84.0	1.9

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amounts of hFSH. The average per

cent recovery for 6 serum pools spiked with hFSH at 3 levels was: $97.3 \pm 3.5\%$.

Dilution test (linearity)

Three samples were measured in a series of dilution with zero-standard. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$$Y = 1,0222X + 0,04 \quad R = 0,9998 \quad n = 12$$

Expected Values

male: 1.0 - 10.5 mIU/ml

female:

ovulatory peak: 4.0 - 13.5 mIU/ml

pre- and postovulatory: 0.6 - 9.5 mIU/ml

postmenopausal: 30 - 135 mIU/ml

It is recommended that each laboratory determine a reference range for its own patient population.

Limitations

- The reagents supplied in this kit are optimized to measure hFSH levels in serum.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
- Hemolyzed and lipemic specimens may give false values and should not be used.
- The KIT has no "high-dose hook" effect with hFSH levels up to 100000 mIU/ml. Samples expected to have concentrations greater than the highest standard should be diluted with the S1 (0 mIU/ml) and reassayed.
- The results of this assay should be used in conjunction with other pertinent clinical information.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Addition of wash buffer.** For the addition of wash buffer the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions and warnings

Radioactivity

This kit contains radioactive material. Receipt, acquisition, possession, or use of radioactive materials are subject to

regulations, and a licence of (inter)national authorizing bodies. It is the responsibility of the user to ensure that local regulations or codes of practice are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1), Hepatitis B surface Antigen (HBsAg) and Treponema antibody.










Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.

All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Bovine components originate from countries where bovine spongiform encephalopathy has not been reported. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Some components contain sodium azide as an antimicrobial agent. Dispose the waste by flushing it with copious amounts of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 68 mg.

	Use by date	CONTROL	Control
	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risks	TRAC	Tracer
	Consult instructions for use	WASHB	Wash buffer
	In vitro diagnostic medical device		Temperature limitation Store between 2-8°C
	Manufacturer		Radioactive Material
REF	Catalogue number		



Website: <http://www.izotop.hu>

Technical e-mail: immuno@izotop.hu

Commercial e-mail: commerce@izotop.hu

IZOTOP

INSTITUTE OF ISOTOPES Ltd.

1535 Budapest. Pf.: 851.

Tel.: (36-1)392-2577, Fax: (36-1)395-9247

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