



FSH IRMA Kit

REF 20 240250

Σ 250



WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

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INTENDED USE

The FSH IRMA has been designed for the quantitative *in vitro* diagnostic measurement of human follicle stimulating hormone (FSH) in serum or plasma.

PRINCIPLES OF THE IRMA

The IRMA is a double antibody immunoradiometric assay system. The sample antigen is "sandwiched" between the ¹²⁵I labelled tracer antibody and the antibody coated magnetisable polystyrene particles (Solid Phase). After incubation the resultant "sandwich" is sedimented, decanted and washed to remove unbound ¹²⁵I labelled antibody. The tubes containing the sedimented "sandwich" are then counted using a gamma counter. The concentration of the analyte is directly proportional to the bound radioactivity of the sandwich. Counts from the calibrators are plotted and sample are read from the constructed calibrator curve.

REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 250 tests. The kit and all its components should be stored at 2-8°C until the listed expiry dates.

FSH: Tracer

1 vial Cat # FSI1
65 mL containing ¹²⁵I labelled anti-FSH (19.3µCi) in BSA PBS buffer, non-immune animal serum and an orange dye. Contains sodium azide, 0.1% w/v. Ready to use.

FSH: Solid Phase

1 vial Cat # FSA1
65 mL containing anti-FSH antibody coupled to magnetisable polystyrene particles in BSA PBS buffer and a blue dye. Contains sodium azide, 0.1% w/v. Resuspend gently before use.

Wash Concentrate

1 vial Cat # CGW1
10 mL of a 15 x concentrated wash solution. Contains sodium azide, 1.5 % w/v. To be diluted before use.

FSH: Calibrators

9 vials Cat # FSS1-9
2.0 mL each in human serum. Contains sodium azide, 0.1% w/v. Ready to use.

PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

Specimens and Calibrators

The source material of the calibrators has been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and has been found to be non-reactive for all. However it is recommended that all specimens be handled as if capable of transmitting infectious disease.

Preservatives

The kit contains sodium azide as a preservative. As reagents contain a potentially toxic preservative, care should be taken in handling, to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

Radioactive Material

The tracer contains radioactive material.

SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Specimens can be either serum or plasma collected in a manner appropriate for laboratory testing. Serum is preferred, however the anticoagulant heparin or EDTA can be employed without sacrificing accuracy.

Avoid grossly haemolytic, lipaemic and turbid specimens. Specimens can be stored at 2-8°C for up to 48 hours. Specimens held for longer should be stored at or below -20°C. Specimens should not be frozen and thawed repeatedly. Thawed specimens should be checked for flocculent matter and mixed by inversion just prior to testing.

Turbid specimens or specimens containing particulate matter should be centrifuged prior to use.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- * Distilled or deionised water
- * Disposable plastic test tubes 12 x 75 mm
- * Precision pipettes
- * Repeating pipettes
- * Timer
- * Vortex mixer
- * Water bath (37°C ± 2°C)
- * Magnetic Racks or Refrigerated centrifuge capable of 1500 x g
- * Absorbent paper
- * Gamma counter

PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use.

Do not use magnetic stirrer to resuspend solid phase reagent.

Contamination of reagents will lead to poor performance. A calibrator curve should be run with each assay.

Specimens suspected of having concentrations above the top calibrator should be diluted in zero calibrator before assay. All assay steps should be performed without interruption.

Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed.

The gamma counter and all pipettes used should be calibrated appropriately before use.

Washing

The efficiency of the wash step is vital for good precision.

Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

ASSAY PROCEDURE

Preparation of Reagents

Wash Solution

Dilute the wash concentrate 1 in 15 with deionised water. The wash solution can be stored at room temperature (20-25°C) for 6 months.

Calibrators

Mix the vials by gentle inversion. The concentrations are stated on each vial label. The calibrator can be stored at 2-8°C.

Protocol

1. Set up and label a sufficient number of test tubes in duplicate for the Total Counts (TC), calibrators, controls and patient samples.
2. Pipette 100 µL of sample (calibrator, control, specimen) into the appropriately labelled tubes.
3. Pipette 250 µL of FSH Tracer (yellow) into all tubes. Set TC tubes aside.
4. Resuspend the FSH Solid Phase (blue-green) by swirling and repeated inversion of the contents of the bottle until no sediment can be seen on the bottom - do not shake this reagent vigorously.
5. Pipette 250 µL of FSH Solid Phase (blue-green) into all tubes except TC.

6. Vortex tubes gently and then incubate for 1 hour at 37°C.
7. Separation of the sandwich from unbound antibody label may be achieved by using either magnetic separation or centrifugation.

A. Magnetic Separation

- a) Place tubes into magnetic separation rack and ensure that all tubes are in contact with the magnetic baseplate. Leave for 15 minutes. Precision can be improved by increasing the time of sedimentation to 20 minutes.
- b) After separation do not remove rack from magnetic baseplate. Decant the supernatant and, keeping the magnetic baseplate inverted, allow tubes to drain onto absorbent paper for 2 minutes.

- c) Remove the rack from its magnetic baseplate. Wash the tubes by adding 500 µL wash solution to all tubes. Vortex, sediment on magnetic baseplate, decant, and blot as above.

OR

B. Centrifugation

- a) Centrifuge all tubes for 5 minutes at 1500 x g at 4°C. Decant the supernatant and allow tubes to drain onto absorbent paper for 2 minutes.
- b) Wash the tubes by adding 500 µL wash solution to all tubes. Vortex, centrifuge, decant and blot as above.
8. Count all tubes for one minute in a gamma counter. Record the cpm of each tube.
9. Calculate results.

CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction.

1. Determine the average CPM for duplicate tubes.
2. Plot the calibrator curve on a semi-log or log-linear graph paper using either of the methods below;

Method 1

Use the following formula to calculate %B/T:

$$\%B/T = \frac{\text{cpm (Sample)}}{\text{cpm Total}} \times 100$$

Plot %B/T on the y axis versus the stated concentrations of the calibrators.

Method 2

Plot the cpm on the y axis versus the stated concentrations of the calibrators.

3. Read sample values directly off the calibrator curve as IU/L.

MODEL CALCULATIONS

ID	Ave cpm	%B/T	FSH IU/L 2nd IRP
Totals	113876		
0	89	0.08	
1.0	289	0.25	
2.5	615	0.54	
5.0	1096	0.96	
10	2331	2.05	
25	5895	5.18	
50	12041	10.57	
100	24138	21.41	
250	50736	44.55	
Sample 1	1410	1.24	6.12
Sample 2	6239	5.48	26.24

CALIBRATION

The calibrators supplied in this kit are calibrated and labelled in IU/L, referenced to the 2nd IRP FSH/LH (2nd IRP 78/549).

LIMITATIONS

Serum specimens showing gross haemolysis, gross lipaemia, or turbidity may give false results.

Specimens that contain appreciable background radioactivity should not be used. Any suspect specimens should be screened for radioactivity before performing the assay and should be held until the radioactivity has decayed, or a new specimen requested.

EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. The following reference range was obtained by assaying serum samples from healthy individuals and is given as a guide only:

Female follicular/ luteal	2 - 12 IU/L
mid-cycle	8 - 22 IU/L
post menopausal	20 - 90 IU/L
Male	1 - 7 IU/L

PERFORMANCE CHARACTERISTICS

Intra-assay Precision

Sample	n	Mean ± 2SD (IU/L)	%CV
A	10	6.9 ± 0.24	3.5
B	10	15.8 ± 0.53	3.4
C	10	43.4 ± 0.36	0.8

Inter-assay Precision

Sample	n *	Mean ± 2SD (IU/L)	%CV
A	10	7.2 ± 0.41	5.7
B	10	16.6 ± 0.64	3.8
C	10	42.8 ± 1.74	4.1

* duplicate

Specificity

Analyte	Concentration Assayed	Apparent FSH Result (IU/L)
hCG	45000 IU/L	not detectable
LH	1000 IU/L	0.40
TSH	1000 mIU/L	0.50

Accuracy

Recovery was calculated by assaying before and after addition of exogenous (x) analyte. 50 µL of sample X was added to 50 µL of each Calibrator.

Sample	FSH (IU/L) Observed	FSH (IU/L) Expected	Recovery %
x (100 µL)	6.2		
x (50 µL) + 50 µL 0	3.1	3.1	100
2.5	4.5	4.4	102
25	14.8	15.6	95
250	118.5	128.1	93

Dilution

A sample was diluted in zero calibrator, assayed and recovery calculated.

Sample	FSH (IU/L) Observed	FSH (IU/L) Expected	% Recovery
Neat	21.3		
1/2	10.7	10.6	100.9
1/4	5.3	5.3	100.0
1/8	2.7	2.7	100.0
1/16	1.4	1.3	107.6

High-dose Hook Effect

Due to the high-dose hook effect characteristic of the assay, samples greater than 1000 IU/L may yield aberrant results, less than that of the kits highest calibrator. Those samples should be diluted with the zero calibrator and reassayed.

Sensitivity

The sensitivity of the assay is typically <0.5 IU/L. The sensitivity is defined as the concentration of analyte which corresponds to the does response variable (cpm) that is two standard deviations greater than the mean dose response variable of 10 replicate determinations of the zero calibrator run in three different assays.

Interference

No interference with analyte recovery was observed for concentrations of haemoglobin up to 250 mg/dL, bilirubin up to 10 mg/dL and triglycerides up to 970 mg/dL.

ORDERING INFORMATION

The FSH IRMA is manufactured by:

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