



FREE α -GLYCOPROTEIN SUBUNIT IRMA KIT

REF 20 222050 **REF** 20 222500

Σ 50 **Σ** 500



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 Free α -Glycoprotein Subunit IRMA has been designed for the quantitative *in vitro* diagnostic measurement of Free α -Glycoprotein Subunit 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 samples are read from the constructed calibrator curve.

REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 50 tests and 500 tests (in parenthesis). The kit and all its components, unopened or opened, should be stored at 2-8°C until the listed expiry dates.

Free α -Glycoprotein

Subunit: Tracer

1 vial REF # AG11
(1 vial REF # AG1260)
27 (260) mL containing ¹²⁵I labelled anti-Free α -Glycoprotein subunit (9.6 μ Ci/96 μ Ci) in BSA PBS buffer, non-immune animal serum and an orange dye. Contains sodium azide, 0.1% w/v. Ready to use.

Free α -Glycoprotein

Subunit: Solid Phase

1 vial REF # AGA1
(1 vial REF # AGA260)
27 (260) mL containing anti-Free α -Glycoprotein subunit 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 REF # CGW1
(2 vials REF # CGW1)
10 mL of a 15 x concentrated wash solution. Contains sodium azide, 1.5% w/v. To be diluted before use.

Free α -Glycoprotein

Subunit: Calibrators

7 vials REF # AGS1-7
(14 vials REF # AGS1-7)
1.0 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 samples 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
- * Precision pipettes
- * Repeating pipettes
- * Vortex mixer
- * Timer
- * 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.

Duplicates are recommended. 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

Exact concentrations determined lot-to-lot are stated on a separate label inside the kit. The calibrators should be stored at -20°C.

Protocol

1. Assemble and label test tubes in duplicate according to the number of tests required. Include Total Counts (TC), calibrators, controls and patient specimens.
2. Pipette 50 μ L of sample (calibrator, control specimen) in duplicate into the appropriate test tubes.
3. Resuspend the Free α -Glycoprotein Subunit 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.
4. Pipette 500 μ L of Free α -Glycoprotein Subunit Tracer (yellow) into all tubes. Set TC tubes aside.

5. Pipette 500 µL of Free α-Glycoprotein Subunit Solid Phase (blue-green) into all tubes except TC.
6. Vortex tubes gently and then incubate for 1 hour at room temperature.
7. Separation of the sandwich from unbound antibody label may be achieved by using 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 in a refrigerated centrifuge 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:

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

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 samples directly off the calibrator curve as IU/L.

MODEL CALCULATIONS

ID	Ave cpm	%B/T	αhCG IU/L
TC	231940		
0	195	0.08	
0.1	458	0.19	
0.5	2312	1.00	
2.5	9356	4.03	
5.0	16407	7.07	
25	58774	25.34	
100	110846	47.79	
Sample 1	1753	0.76	0.39
Sample 2	10259	4.42	2.50

CALIBRATION

The calibrators supplied in this kit are calibrated and labelled in IU/L, referenced to 1st IRP for αhCG 75/569.

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:

Adult Female	
pre menopausal	0.1 - 0.5 IU/L
post menopausal	0.6 - 1.5 IU/L
Adult Male	0.1 - 0.5 IU/L

PERFORMANCE CHARACTERISTICS

Intra-assay Precision

Sample	n	Mean ± 2SD (IU/L)	%CV
1	20	0.35 ± 0.18	5.1
2	20	3.03 ± 0.11	3.9
3	20	37.40 ± 1.29	3.5

Inter-assay Precision

Sample	n *	Mean ± 2SD (IU/L)	%CV
1	20	0.38 ± 0.02	5.3
2	20	2.87 ± 0.15	5.2
3	20	32.50 ± 1.30	4.0

* duplicate

Specificity

Analyte	Concentration Assayed	Apparent αGP (IU/L) Results
LH	250 IU/L	1.80
FSH	250 IU/L	0.53
TSH	250 mIU/L	0.39

Accuracy

Recovery was calculated by assaying before and after addition of exogenous (x) analyte. 25 µL of sample X was added to 25 µL of each calibrator.

Sample	αGP (IU/L) Observed	αGP (IU/L) Expected	Recovery %
x (50 µL)	1.46		
x (25 µL) + 25 µL	0	0.73	101
	0.1	0.77	99
	0.5	1.07	109
	2.5	2.00	101
	5.0	3.30	102
	25	14.00	106
	100	50.00	98

Dilution

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

Sample	αGP (IU/L) Observed	αGP (IU/L) Expected	% Recovery
Neat	85.9		
1/2	44.5	42.9	104.0
1/4	21.4	21.5	99.6
1/8	10.1	10.7	94.0
1/16	5.2	5.3	97.0

High-dose Hook Effect

Due to the high-dose hook effect characteristic of the assay, samples greater than 12,000 IU/L may yield aberrant results, less than that of the kit's highest calibrator. Those samples should be diluted with the zero calibrator and reassayed.

Sensitivity

The sensitivity of the assay is typically <0.03 IU/L.

The sensitivity is defined as that concentration of analyte which corresponds to the dose response variable (cpm) that is two standard deviations from 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 *Free α-Glycoprotein Subunit IRMA* is manufactured by:

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