RK-820CTACE210501

Free ßhCG [I-125] IRMA KIT

(REF: RK-820CT)

The ^{125}I-free ßhCG IRMA system provides direct quantitative *in vitro* determination of human Chorionic Gonadotrophin ß-subunit (ßhCG) in human serum. Free ßhCG can be assayed in the range of 0-100 mIU/ml using 50 µl serum samples.

Introduction

Human Chorionic Gonadotrophin (hCG) is a glycoprotein with a molecular weight of 38000, secreted by the trophoblast cells of placenta. It contains two different subunits. The α-subunit is common to all glycoprotein hormones and the ß-subunit is responsible for the immunological and biological specifity. Free forms of both subunits are present in the circulation.

Normal pregnancy is associated with an exponential increase of both holo-hCG and its free ß-subunit until the 10th week. Then concentration decreases gradually. Maximum levels can reach values up to 100 mIU/ml.

The significant elevation of free ßhCG in maternal serum is now considered as a specific and sensitive diagnostic marker of gestational trophoblastic neoplasiae (GTN) including hydatidiform mole (both invasive and non-invasive) and choriocarcinoma. In addition to its use as a screening method, the appearence of free ßhCG after surgical abortion is a good indication of tumour recurrence.

This kit can be used in the risk assessment of Down's Syndrome (Trisomy 21) in combination with other biochemical and ultrasound parameters as specified above, taking also into account other data like maternal age and weight and using a validated software for Down's Syndrome risk assessment (***see Annex***):

Combined test: maternal serum PAPP-A and free-βhCG determination and nuchal translucency (NT) thickness measurement by an ultrasound scan in the first trimester of pregnancy.

Principle of method

The technology uses two monoclonal antibodies of high affinity in an immunoradiometric assay (IRMA) system. This assay is based on a two-steps procedure to eliminate interference from serum protein. In the first stage the serum sample is incubated in streptavidin coated tubes with biotin labelled monoclonal antibody (capture). During 1-hour incubation period with continuous agitation the immuno-complex is developed and immobilized on the reactive surface of test tubes. After incubation tubes are washed. In the second stage ^{125}I labelled monoclonal antibody (signal) is added and it binds to an epitope of the free ßhCG molecule different from that recognised by the unlabelled capture-antibody developing the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich". 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 free ßhCG, the unknown concentration of free ßhCG in patient samples can be determined.

Contents of the kit

1. 1 bottle TRACER (32 ml), ready to use, containing about 740 kBq ^{125}I-anti- ßhCG antibody in buffer with red dye 0.1 % NaN_3.

2. 1 bottle ANTISERUM (21 ml), ready to use, containing capture anti-free ßhCG antibody in buffer with blue dye and 0.1 % NaN_3.

3. 6 vials STANDARD (6 x 0.5 ml), containing (S1-S6) 0, 0.1, 0.5, 2, 10, 100 mIU/ml free ßhCG (calibrated against WHO IRP 75/551) in bovine serum with 0.1% NaN_3.

4. 1 vial CONTROL SERUM 1.0 ml human serum with 0.1% NaN_3. The concentration of the control serum is specified in the quality certificate enclosed.

5. 2 boxes COATED TUBE, Ready to use.
2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

6. 2 bottle WASH BUFFER CONCENTRATE (2 x 20 ml), containing 0.2% NaN_3. See *Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (50, 200, 300 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, adsorbent tissue, gamma counter

Recommended tools and equipment

repeating pipettes (e.g. Eppendorf or else), dispenser with 1.5-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. Hemolyzed and lipemic specimens may give false values and should not be used. Repeated freezing and thawing of specimens must be avoided

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.

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.

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 for each standard (S1-S6), control serum and samples.
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 50 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 antiserum 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.
7. Incubate tubes for 1 hour, shaking 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. Pipette 300 µl of tracer into each tube.
11. 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.
12. Incubate tubes for 1 hour, shaking at room temperature.
13. 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.
14. Return the tube-rack to an upright position, and repeat step-13 one more time
15. Count each tube for at least 60 seconds in a gamma counter.
16. Calculate the free ßhCG 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		**50**		
Control			**50**	
Sample				**50**
Antiserum	**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				
Tracer	**300**	**300**	**300**	**300**
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:

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

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of free ßhCG.
Determine the free ßhCG 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. Automated data processing systems are also available.

Table 2. Typical assay data

Tubes	Count cpm	Mean cpm	B/T%
T	307023 304769	305896	-
S1	275 245	260	0.09
S2	462 445	454	0.06
S3	1257 1215	1236	0.32
S4	4369 4386	4377	1.35
S5	21050 21309	21179	6.84
S6	131921 131102	131512	42.91
C	25652 26316	25984	8.41

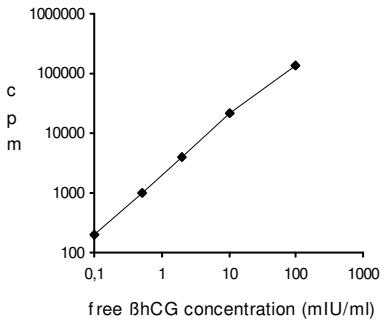

Figure 1: A typical standard curve
(*Do not use to calculate unknown samples!)*

Characterization of assay

Typical assay parameters

NSB/T < 0.3 %

Sensitivity

For the analytical sensitivity 0.02 mIU/ml has been obtained by assaying 15 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.

Hook effect

There is no high dose " hook effect " up to a free ßhCG concentration of 2500 mIU/ml.

Specificity

The capture monoclonal antibody used in this IRMA kit is specific for free ßHCG. No cross reactivity with hFSH, hLH and hTSH can be detected in normal physiological concentrations.

Precision and reproducibility

Four patient samples were assayed in 15 replicates to determine intra-assay precision. To determine inter-assay precision four patient samples were measured in duplicates in 15 independent assays using different kit batches. Values obtained are shown below.

Intra-assay		Inter-assay	
mean (mIU/ml)	CV %	mean (mIU/ml)	CV %
4,93	1.8	4.69	4.1
9,02	1.1	8.63	3.6
14,33	3.4	12.8	5.4
41,38	1.1	38.0	9.0

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of free ßhCG. The average per cent recovery for 3 serum samples spiked with free ßhCG at 5 levels was:
99.2 ± 5.5 (mean ± SD).

Dilution test (linearity)

4 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 = 1.015**x** – 0.0316 R = 0,9995 n =20

Expected Values

Healthy adult (expect pregnant women):
< 0.1 mIU/ml

In the first trimester of pregnancy:

Pregnancy Week	1MOM (mIU/ml)	N
10	51.19	6
11	44.67	163
12	38.9	821
13	34.4	399

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

The results obtained should only be interpreted in the context of the overall clinical picture. None of the *in vitro* diagnostic kits can be used as the one and only proof of any disease or disorder.

Every three months MoM values should be checked and, if necessary, recalculated.

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 1.5-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.

Precaution

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials 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) and Hepatitis B surface Antigen (HBsAg).
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*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 93 mg.

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

CE 1011

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Annex to the Instructions for Use

In vitro kits for Down's Syndrome risk assessment

This kit has been validated for the risk assessment of 21 Trisomy, using the following kits and software:

Kit / software name	Code	Manufacturer
PAPP-A IRMA	RK-4 CT	Institute of Isotopes Ltd.
Free βhCG IRMA	RK-820CT	Institute of Isotopes Ltd.
AFP IRMA	RK-800 CT	Institute of Isotopes Ltd.
hCG RIA	RK-770 CT	Institute of Isotopes Ltd.
Unconjugated Estriol RIA	RK-3 CT	Institute of Isotopes Ltd.
Active Inhibin-A ELISA	DSL-10-28100	DSL - Beckman Coulter
αlpha - Antenatal Screening Software for Down's Syndrome and Neural Tube Defects.		Logical Medical Systems Ltd.
*The kits and software listed before are CE marked. They can be used together for the risk assessment of Trisomy 21, according to the 98/79 EC IVD directive and based on the conformity assessment performed by an authorized notified body (**CE1011**).*		

Resumed results of validation, regarding efficiency of risk assessment:

Sensitivity

Screening method	Number of tests performed	Positive cases with the test		Cases confirmed by cytogenetic test		Fals positive cases	
		No.	%	No.	% of positive tests	No.	%
Combined Test	1389	42	3,02	2	4,76	40	2,88
Quadruple Test	539	30	5,57	1	3,33	29	5,38
Integrated Test	1741	47	2,70	3	6,38	44	2,53

Specificity

Screening method	Number of tests performed	Negative cases	Negative cases with the test		Fals negative cases
			No.	%	No. (%)
Combined Test	1389	1387	1345	96,97	1 (0,07)
Quadruple Test	539	538	508	94,42	0
Integrated Test	1741	1738	1691	97,30	0

Literature: see www.izotop.hu

CE 1011

WEB site: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

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