RK-770CTACE110601

hCG [^{125}I] RIA KIT

(REF: RK-770CT)

The ^{125}I-hCG RIA system provides direct quantitative *in vitro* determination of human Chorionic Gonadotrophin (hCG) in human serum. hCG can be assayed in the range of 0-250 IU/ml using 25 µl serum samples.

Introduction

hCG appears in the sera of pregnant women five days after the implantation of blastocyst and its concentration continually increases until the third month of the pregnancy. The maximum concentration can reach values up to 100 IU/ml. Then the hormone level drops to 25 IU/ml and stays around this value until the last trimester.

Elevated hCG concentrations can be seen in the case of trophoblastic and nontrophoblastic neoplasia, and choriocarcinoma.

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***):

1. **Triple test**: maternal serum AFP, hCG and unconjugated Estriol determination in the second trimester of pregnancy.
2. **Quadruple test**: maternal serum AFP, hCG, unconjugated Estriol and Inhibin-A determination in the second trimester of pregnancy.
3. **Integrated test**: maternal serum PAPP-A determination and nuchal translucency (NT) thickness measurement by an ultrasound scan in the first trimester of pregnancy and maternal serum AFP, hCG, unconjugated Estriol and Inhibin-A determination in the second trimester.

Principle of method

This assay is based on the competition between unlabelled hCG and fixed quantity of ^{125}I-labelled hCG for limited number of binding sites on hCG specific antibody. Allowing to react a fixed amount of tracer and antibody with different amounts of unlabelled ligand the amount of tracer bound by the antibody will be inversely proportional to the concentration of unlabelled ligand.

During 1-hour of incubation period with continuous agitation, immuno-complex is immobilized on the reactive surface of test tubes. After incubation the reaction mixture is discarded, and the radioactivity is measured in a gamma counter.

The concentration of antigen is inversely proportional to the radioactivity measured in test tubes. By plotting binding values against a series of calibrators containing known amount of hCG, a calibration curve is constructed, from which the unknown concentration of hCG in patient samples can determined.

Contents of the kit

1. 1 bottle ^{125}I-TRACER (55 ml), ^{125}I-labelled hCG in buffer with red dye and 0.1 % NaN_3, containing <260 kBq.

2. 1 bottle ANTISERUM (55 ml), containing anti-ßhCG IgG in buffer with blue dye and 0.1 % NaN_3.

3. 6 vials STANDARD (6 x 0.4 ml), containing (S1-S6) 0; 7; 15; 40; 100; 250 IU/ml hCG (calibrated to WHO 4th IRP 75/589) in serum with 0.1% Kathon CG.

4. 1 vial CONTROL SERUM, 0.4 ml human serum with 0.1% Kathon CG.

The concentration of the control serum is specified in the quality certificate enclosed.

5. 2 boxes COATED TUBE, 2x50 pcs, 12x75 mm packed in plastic boxes.

Quality certificate

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (25 and 500 µl), shaker, plastic foil, absorbent tissue, gamma counter.

Recommended tools and equipment

Repeating pipettes.

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. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens.

Preparation of reagents, storage

Store the 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 (min. for an hour).
2. Label coated tubes in duplicate for each standard (S1-S6), control serum (C) and samples (M). Optionally, label two test tubes for total count (T).
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 25 µl each of standards, control and samples into the properly labelled tubes.
5. Pipette 500 µl of tracer into each tube.
6. Pipette 500 µl of antiserum into each tube except T.
7. Fix the test tube rack firmly onto the shaker plate. Seal all tubes with a plastic foil. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
8. Incubate tubes for 1 hour at room temperature.
9. Aspirate or 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.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the hCG concentrations of the samples as described in calculation of 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 (optionally) the percent $B_0/T\%$ for zero standard (S_1) by using the following equation:

$$B_0/T\% = \frac{S_1\ (cpm)}{T\ (cpm)} \times 100$$

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

$$B/B_0(\%) = \frac{S_{2\text{-}6} / C / M_x\ (cpm)}{S_1(cpm)} \times 100$$

For simplicity, these values are uncorrected for non-specific binding (NSB). This is enabled by low NSB being less than 3 % of total counts.

Using semi-logarithmic graph paper plot B/B_0 (%) for each standard versus the corresponding concentration of hCG. Figure 1 shows a typical standard curve. Determine the hCG 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 1. Assay Protocol, Pipetting Guide (*all volumes in microlitres*)

	T	S1-S6	C	M
Standard		**25**		
Control			**25**	
Samples				**25**
Tracer	**500**	**500**	**500**	**500**
Antiserum		**500**	**500**	**500**
Shake for 1 hour at room temperature				
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

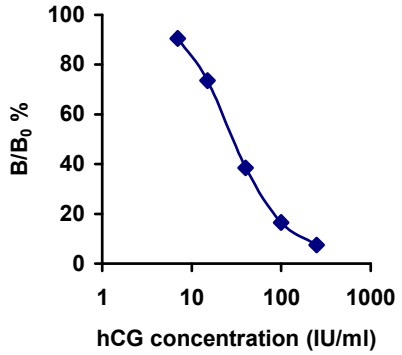


Figure 1
A typical standard curve
(Do not use to calculate sample values!)

Table 2. Typical assay data

Tubes	Count cpm	Mean cpm	B0/T %	B/B0 %
T	103449 102245	102847		
S_1	72967 72870	72918	70.9	100.0
S_2	65706 66325	66016	64.2	90.5
S_3	54584 52606	53595	52.1	73.5
S_4	28614 27692	28153	27.4	38.6
S_5	12465 11788	12127	11.8	16.6
S_6	5512 5303	5408	5.3	7.4
C	33432 33956	33694	32.8	46.2

Characterization of assay

Typical assay parameters

B_0/T 67 ± 8 %
ED-50: 24.8 – 32.8 IU/ml

Sensitivity

The analytical sensitivity of this assay is 1.25 IU/ml, as obtained by measuring 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.

Specificity

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

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amounts of hCG. The average per cent recovery for 6 serum pools spiked with hCG at 5 levels was: 98.51 ± 4.6 (mean ± SD).

Precision and reproducibility

7 patient samples were assayed in 15 replicates to determine intra-assay precision. To determine inter-assay precision 7 patient samples were measured in duplicates in 15 independent assays by 3 operators using different kit batches. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean (IU/ml)	CV %	Mean (IU/ml)	CV %
5.06	6.5	4.67	8.3
17.40	5.5	17.17	3.0
32.93	2.9	32.57	3.7
50.49	3.9	49.07	3.9
80.13	2.7	78.38	3.6
102.31	3.0	102.01	3.7
197.36	7.2	203.19	4.4

Dilution test (linearity)

6 samples were serially diluted with zero-standard and measured according to kit protocol. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

y = 1.0095**x** + 0.7158 R = 0,9996 n =22

Expected Values in the Second Trimeser of Pregnancy

Each laboratory should establish its own range of expected values. The values presented below can be used as a guideline only.

Weeks of pregnancy	1MOM (IU/ml)	N
15	36.44	1013
16	28.1	913
17	23.5	123

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) **Source of error!** Do not use a shaker in which some tubes can be exposed to heating. Do not place the shaker directly by an air conditioning or heating device or by an open window. Any differences in temperature between tubes during incubation can lead to serious measuring errors.

Additional information

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

Precaution

Radioactivity

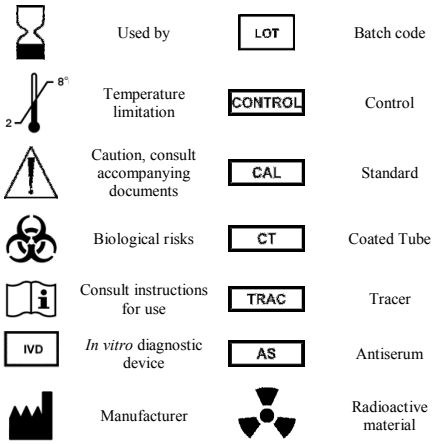
This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

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

Some 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 110 mg. Some components contain Kathon CG as an antimicrobial agent. The total Kathon CG present in each pack is 2.8 mg.



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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

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WEB site: http://www.izotop.hu
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