RK-770CTACE070611

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.

The current RIA system is particularly matched to the direct determination of gestational βhCG levels, neoplastic βhCG can not be measured.

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 a 1-hour 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 against 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 (e.g., Eppendorf, or else)

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.

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 the percent $B_0/T\%$ for zero standard (S_1) by using the following equation:

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

$B_0/T\%$ is an optional quality control parameter unnecessary for determination of sample concentrations.

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 count.

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				

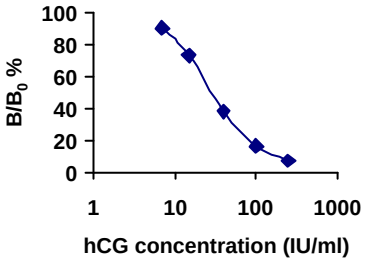


Figure 1.
A typical standard curve
(Do not use to calculate sample values!)

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

For the analytical sensitivity 1,25 IU/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.

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.

Precision

7 patient samples were assayed in 15 replicates to determine intra-assay precision. Values obtained are shown below.

Sample	Number of replicates	Mean value	SD	CV %
1	15	5.06	0.33	6.5
2	15	17.40	0.96	5.5
3	15	32.93	0.97	2.9
4	15	50.49	1.98	3.92
5	15	80.13	2.12	2.70
6	15	102.31	3.08	3.0
7	15	197.36	14.30	7.2

Reproducibility

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.

Sample	Number of runs	Mean value	SD	CV %
1	15	4.67	0.39	8.3
2	15	17.17	0.51	3.0
3	15	32.57	1.20	3.7
4	15	49.07	1.92	3.9
5	15	78.38	2.82	3.6
6	15	102.01	3.74	3.7
7	15	203.19	8.96	4.4

Recovery

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

Dilution test (linearity)

6 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 = 1.0095**x** + 0.7158 R = 0,9996 n =22

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.

Limitations

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

Do not use lipemic, hemolyzed or turbid specimens.

It is recommended that each laboratory establish its own reference intervals.

Without linked and validated software this kit is NOT intended to be used for the risk evaluation of trisomy 21.

Precaution

Radioactivity

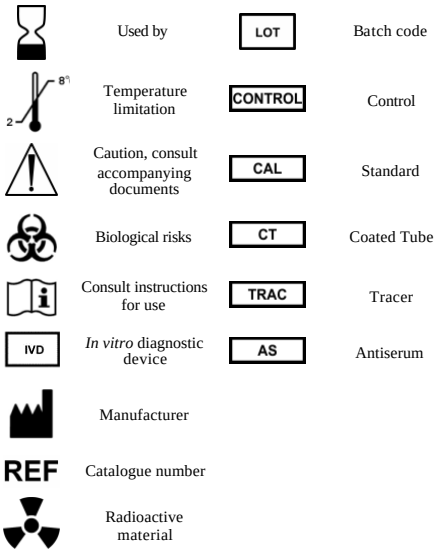
This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

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 110 mg.



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