RK-83CTACE190401

hCalcitonin [I-125] IRMA KIT

(REF: RK-83CT)

The ^{125}I-hCalcitonin IRMA system provides direct quantitative *in vitro* determination of human calcitonin in human serum. Calcitonin can be assayed in the range of 0-2000 pg/mL using 100 µL serum samples. Each kit contains material sufficient for 100 tests, permitting the construction of one standard curve and assay of 42 unknowns and 2 controls in duplicate.

Introduction

Calcitonin (MW 3.4 kDa) is primarily secreted by the parafollicular C-cells of the thyroid gland. The mature peptide hormone comprises 32 amino acid residues. Calcitonin exerts its biological effect by acting on its target organs: bone, kidney and the gastrointestinal tract. The physiological role of calcitonin in bone metabolism is not fully understood and is still under investigation. It is well established that abnormally elevated levels of calcitonin are characteristic of thyroid C-cell hyperplasia and medullary thyroid carcinoma (MTC). MTC represents 5-10 % of all thyroid cancer and exists in either familial or sporadic form. The determination of Calcitonin in human serum is recommended for the diagnosis and follow-up of MTC and for diagnosis of preclinical cases of the familial forms of MTC.

In the blood the apparent calcitonin-like immunoreactivity is contributed by various calcitonin-related species, including the monomeric, dimeric and polymeric forms, as well as fragments and precursors of the parent hormone.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The ^{125}I labelled signal-antibody binds to an epitope of the Calcitonin molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During the overnight incubation period the immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes are 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 amounts of Calcitonin, the unknown concentration of Calcitonin in patient samples can determined.

Contents of the kit

1. 1 bottle of TRACER, Ready to use. 11 mL per vial, containing < 740 kBq ^{125}I-signal and biotin-capture antibody in buffer with red dye and 0.1 % NaN_3.

2. 6 vials of STANDARDS (S0-S5, 6 x 1 mL) Freeze-dried, in equine serum with 0.1% Kathon-CG. The exact concentrations are indicated on each vial and in the quality certificate enclosed. (Calibrated with WHO international standard 89/620). See *Preparation of reagents.*

3. 2 vials of CONTROL SERA, (CI-CII, 2 x 1 mL). Freeze-dried, in human serum with 0.1% Kathon-CG. See *Preparation of reagents*. The acceptance ranges of the controls are specified in the quality certificate enclosed.

4. 1 vial of DILUENT, ready to use. 2 mL per vial, equine serum with 0.1% Kathon-CG.

5. 2 boxes of COATED TUBES, Ready to use.

2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

6. 1 bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.2% NaN_3. See *Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

- common laboratory equipment
- 100 µL precision micropipette
- 100 µL repeating pipette
- 2000 µL repeating pipette or dispenser
- plastic foil to cover tubes
- absorbent tissue
- gamma-counter

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.

Preparation of reagents, storage

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. After dilution, store at 2-8°C until expiry date of the kit.

Add 1 mL distilled water to the lyophilized standards and control sera. Mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved and allow the solution to equilibrate at room temperature for at least 20 minutes. **For further use, reconstituted standards and controls have to be stored below -20°C until the 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.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Equilibrate all reagents and samples to room temperature before use. Homogenize by gentle mixing to avoid foaming.
2. Label coated tubes in duplicate for each standard (S0-S5), control serum (CI, CII) and samples.
3. Pipette 100 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.
4. Pipette 100 µL of tracer into each tube.
5. Gently vortex all tubes. Seal all tubes with a plastic foil.
6. Incubate tubes for 16-24 hours at room temperature.
7. Add 2,0 mL diluted wash buffer to each tube and 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.
8. Return the tube-rack to an upright position and repeat Step-7 two times more.
9. Count each tube for at least 60 seconds in a gamma counter.
10. Calculate the Calcitonin concentration 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		**100**		
Control			**100**	
Sample				**100**
Tracer	**100**	**100**	**100**	**100**
Vortex, incubate for 16-24h 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				
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:

$$B/T(\%) = \frac{S_{1\text{-}5} / C / M_x \text{ (cpm)} - S0 \text{ (cpm)}}{T(\text{cpm})} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of calcitonin.

Determine the calcitonin 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	Mean cpm	B/T%
T	303 022	-
S0	182	0.06
S1	1 459	0.42
S2	4 759	1.51
S3	15 195	4.95
S4	46 921	15.4
S5	143 589	47.3
CI	2 804	0.87
CII	9 714	3.15

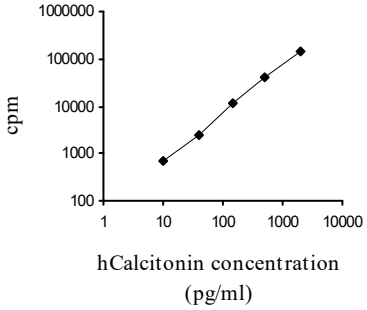

Figure 1: A typical standard curve
(*Do not use to calculate unknown samples)*

Characterization of assay

Sensitivity

For the analytical sensitivity, 0.5 pg/mL has been obtained by assaying 20 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.

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the CLSI guidelines, document EP17.

Limit of Blank (LoB): 0.65 pg/mL

Limit of Detection (LoD): 1.2 pg/mL

Limit of Quantitation (LoQ): 2.0 pg/mL

The functional sensitivity is equal to the Limit of Quantitation (LoQ).

Precision and reproducibility

To determine intra-assay precision 7 samples were assayed in 20 replicates. To determine inter-assay precision 7 samples were measured in 20 independent assays by 3 operators using different kit batches. Values obtained are shown below.

Sample	intra-assay		inter-assay	
	Mean (pg/mL)	CV%	Mean (pg/mL)	CV%
1	0.9	17.9	1.8	21.9
2	23.6	2.7	24.9	5.1
3	25.3	3.2	28.2	6.0
4	161.2	1.6	170.6	6.2
5	180.8	4.4	198.0	6.6
6	348.3	1.7	364.4	6.4
7	782.3	1.4	812.4	6.4

Recovery

Recovery was defined as the measured increase expressed as percent of expected increase upon spiking serum samples with known amount of Calcitonin. The average percent recovery for 4 serum samples spiked with Calcitonin at 3 levels was 101-122 %.

Specificity

The monoclonal antibodies used in this IRMA kit are specific for human Calcitonin. Interference of human Procalcitonin in the assay cannot be detected for Procalcitonin concentrations ≤ 80 ng/mL.

Linearity

Five individual serum samples were serially diluted with human serum with low Calcitonin concentration and measured according to kit protocol. Mean recovery after dilution was 110%. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$Y = 1.1394X - 1.5616 \quad R^2 = 0.9951 \quad n = 20$

Hook effect

The KIT has no "high-dose hook" effect with Calcitonin levels up to 300 000 pg/mL. Samples expected to have concentrations greater than the highest standard should be diluted with the dilution serum and reassayed. Sequential dilutions 1:10 are recommended.

Expected Values

Based on the measurement of 607 presumably healthy adult serum samples (304 female and 303 male), the expected range of Calcitonin is **0 - 10 pg/mL** (99.8% of samples).
It is recommended that each laboratory determine a reference range for its own patient population.

Limitations

- The reagents supplied in this kit are optimized to measure Calcitonin levels in human serum.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
- Hemolyzed and lipemic specimens may give false values and should not be used.
- The results of this assay should be used in conjunction with other pertinent clinical information.
- In some pathological situations Calcitonin could be elevated without any diagnostic or prognostic value, for example in some cases of hypercalcemia, renal insufficiency, hypergastrinemia and acute pancreatitis.

Procedural notes

The non-respect of the instructions in this insert may affect results significantly.
Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

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.

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 antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody 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 infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials.*
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated 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 51 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C.
Shelf-life: 67 days from availability.

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

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