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Calcitonin ultra sensitive ELISA

Enzyme immunoassay for the measurement of human Calcitonin (CT) in serum.

REF **IB79148**

Σ **96**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

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CONTENTS

1. INTENDED USE

Immunoenzymetric assay for measurement of human Calcitonin (CT) in serum.
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2. PRINCIPLES OF THE METHOD

The Calcitonin ultra sensitive ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. Calibrators and samples react with the capture monoclonal antibody (MAb 1) coated on microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAb 1 – human CT – MAb 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB ready for use) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the Calcitonin concentration. A calibration curve is plotted and Calcitonin concentration in samples is determined by interpolation from the calibration curve.

3. REAGENTS PROVIDED

Reagents	96 tests Kit	Reconstitution
SORB MT Microtiterplate with 96 anti CT (monoclonal antibodies) coated breakable wells	96 wells	**Ready** for use
ENZ CONJ 50x Conjugate: HRP labelled anti-CT (monoclonal antibodies) in Stabilizing Buffer	1 vial 0.125 ml	**Dilute** 50 x with conjugate buffer
ENZ CONJ DIL Conjugate buffer: TRIS-Maleate buffer with bovine serum albumin, EDTA and thymol	1 vial 6 ml	**Ready** for use
CAL 0 – 5 LYO Calibrator N = 0 to 5 (see exact values on QC data sheet) in CT-Free human serum	6 vials lyophil.	**Add** 0.5 ml distilled water
SAM DIL LYO CT free human serum (to be used for samples dilution) with thymol	1 vial lyophil.	**Add** buffer (see reconstitution volume on the QC data sheet)
BUF Buffer (serum free): borate buffer	1 vial 8 ml	**Ready** for use
WASH SOLN 200x Wash Solution (Tris-HCl)	1 vial 10 ml	**Dilute** 200 x with distilled water (use a magnetic stirrer).
CONTROL 1 & 2 LYO Controls - N = 1 or 2 in human serum with gentamycin	2 vials lyophil.	**Add** 0.5 ml distilled water
SUB TMB Chromogenic TMB solution (Tetramethylbenzydine)	1 vial 12 ml	**Ready** for use
STOP SOLN Stop Solution: HCl: 1N	1 vial 12 ml	**Ready** for use

Note: 1. CT free human serum is to be used for samples dilution.
2. 1 pg of our reference preparation is equivalent to 0.19 µIU NIBSC 89/620.

4. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. High quality distilled water
2. Pipettes for delivery of: 50 µl, 100 µl,, 500 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Washer for microtiterplate
6. Microtiterplate reader capable of reading at 450 nm and 650 nm (bichromatic reading).

5. REAGENT PREPARATION

A. Calibrators : Reconstitute the calibrators with 0.5 ml distilled water.

B. Controls : Reconstitute the controls with 0.5 ml distilled water.

C. Working Wash solution : Prepare an adequate volume of Working Wash solution by adding 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

D. CT Free Serum: Reconstitute the CT Free Serum with the amount of Buffer as indicated on the QC data sheet. Allow it to remain undisturbed until completely dissolved, and then mix well by gentle inversion.

E. Working anti-CT-HRP conjugate: Prepare an adequate volume of conjugate solution by adding for example :40 µl of the 50 x concentrated anti-CT-HRP conjugate to 2 ml of conjugate buffer. Use a vortex to homogenize. Extemporaneous preparation is recommended.

6. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused wells must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, calibrators, controls and CT free serum are very unstable and should be frozen immediately after use and kept at –20°C for 3 months. Only one freeze thawing cycle is allowed, discard the calibrators, controls and CT free serum after the second use.
- The concentrated Wash Solution is stable at 18-25°C until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the concentrated conjugate (50x) is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- The Working anti-CT-HRP conjugate is stable for 1 week at 4°C.
- The chromogenic TMB solution and the Stop Solution are stable at 2°C to 8°C until the expiry date.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

7. SAMPLE COLLECTION AND PREPARATION

- Serum must be kept at 2 - 8°C.
- If the test is not run within 24 hours, storage in aliquots at -20°C is recommended. Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at 18-25°C. It is recommended to vortex the samples before use.
- Do not use haemolysed samples.
- Do not use lipemic samples.

8. PROCEDURE

A. Handling notes

- Do not use the kit or components beyond expiry date.
- Do not mix materials from different kit lots.
- Thoroughly mix all reagents and samples by gentle agitation or swirling.
- Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.
- Use a clean plastic container to prepare the Wash Solution.
- In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
- For the dispensing of the Chromogenic Solution and the Stop Solution avoid pipettes with metal parts.
- High precision pipettes or automated pipetting equipment will improve the precision.
- Respect the incubation times.
- Prepare a calibration curve for each run, do not use data from previous runs.
- The chromogenic solution should be colourless. If a dark blue colour develops within a few minutes after preparation, this indicates that the preparation is unusable and must be discarded.
- Dispense the Chromogenic Solution within 15 minutes following the washing of the microtiterplate.
- During incubation with Chromogenic Solution, avoid direct sunlight on the microtiterplate.

B. Procedure

1. Select the required number of wells for the run. The unused wells should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the wells into the holding frame.
3. Pipette 100 µl of each Calibrator, Control and Sample into the appropriate wells.
4. Pipette 50 µl of Working anti-CT-HRP conjugate into all the wells.
5. Incubate for 18 ± 1 hour at 2-8°C.
6. Aspirate the liquid from each well.
7. Wash the plate 3 times by:
   - Dispensing 0.4 ml of Wash Solution into each well
   - Aspirating the content of each well
8. Pipette 100 µl of the Chromogenic solution into each well within 15 minutes following the washing step.
9. Incubate the microtiterplate for 30 minutes at 18-25°C avoiding direct sunlight.
10. Pipette 100 µl of Stop Solution into each well.
11. Read the absorbances at 450 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results as described in section 10.

9. CALCULATION OF RESULTS

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. Plot the OD values (ordinate) for each calibrator against the corresponding concentration of Calcitonin (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4 parameter logistic function curve fitting is recommended.

10.PERFORMANCE AND LIMITATIONS

A. Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations above the average OD at zero binding, was 0.7 pg/ml.

B. Specificity

Some potentially interfering hormones have been tested in this assay. At concentrations up to 100 ng/ml, none of the following hormones showed significant interference :

- CGRP
- Salmon-calcitonin
- PDN 21
- Procalcitonin N terminal.

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	**N**	**<X> ± SD (pg/ml)**	**CV (%)**	**Serum**	**N**	**<X> ± SD (pg/ml)**	**CV (%)**
A	19	43.0 ± 0.75	1.7	A	8	44.6 ± 2.1	4.9
B	19	133.7 ± 5.2	3.9	B	8	136.3 ± 8.1	6

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Added CT (pg/ml)	**Recovered CT (pg/ml)**	**Recovery (%)**
327.7	340.6	104
160.7	159.3	99
80.5	80.4	99
48.3	50.8	105

DILUTION TEST

Sample	**Dilution**	**Theoretical Concent. (pg/ml)**	**Measured Concent. (pg/ml)**
Serum 1	1/1	-	300.6
	1/2	150.3	157.9
	1/4	75.1	75.5
	1/8	37.6	45.7
	1/16	18.8	25.2
	1/32	9.4	12.1
	1/64	4.7	5

Samples were diluted with CT Free Serum.

E. Hook effect

A sample spiked with CT up to 480000 pg/ml gives higher OD's than the last calibrator point.

11.INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the QC data sheet, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls that contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

12.REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

Normal values
84 samples from normal subjects obtained values below 11 pg/ml.

13.PRECAUTIONS AND WARNINGS

Safety
For research use only.
The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum samples should be in accordance with local safety procedures.
All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
Avoid any skin contact with all reagents, Stop Solution contains HCl, the chromogenic solution contains TMB and H_2O_2. In case of contact, wash thoroughly with water.
Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

14.SUMMARY OF THE PROTOCOL

	CALIBRATORS (µl)	**SAMPLE(S) CONTROLS (µl)**

	CALIBRATORS (µl)	**SAMPLE(S) CONTROLS (µl)**
Calibrators (0-5) Controls, Samples Working Anti-CT-HRP conjugate	100 - 50	- 100 50
Incubate for 18 ± 1 hours at 2 – 8°C. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash Solution and aspirate.		
Chromogenic TMB Solution	100	100
Incubate for 30 min at room temperature		
Stop Solution	100	100
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm)		

SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta