DRG® Procalcitonin ELISA (EIA-5291)

Revised 2 May 2014 rm (Vers. 2.1) **RUO**

This kit is intended for Research Use Only.

Not intended for diagnostic purposes.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The Human Procalcitonin ELISA is a sandwich enzyme immunoassay for measurement of human procalcitonin.

Features

- It is intended for research use only
- The total assay time is less than 4.5 hours
- The kit measures procalcitonin in serum and urine
- Assay format is 96 wells
- Standard is recombinant protein based
- Quality Controls are human serum based
- Components of the kit are provided ready to use, concentrated or lyophilized

2 STORAGE, EXPIRATION

Store the complete kit at 2 °C - 8 °C. Under these conditions, the kit is stable until the expiration date (see label on the box).

3 INTRODUCTION

Procalcitonin (PCT) the precursor of the hormone calcitonin, is a 116 amino acid protein with a molecular mass of 13 kDa. It undergoes successive cleavages in the neuroendocrine cells of the thyroid to form three distinct molecules; calcitonin (32 amino acids); katacalcin (21 amino acids) and N-terminal fragment called aminoprocalcitonin (57 amino acids). Procalcitonin belongs to a group of related proteins including calcitonin gene-related peptides I and II, amylin, adrenomodulin and calcitonin (CAPA peptide family). Synthesis of procalcitonin is regulated gene CALC-1. Under normal metabolic conditions procalcitonin is present in the C-cells of the thyroid gland. The level of procalcitonin in the blood of healthy individuals is low. The risk of local bacterial infection occurs when the value of procalcitonin exceeds 0.25 ng/ml. The risk of systemic bacterial infection occurs when the value of procalcitonin exceeds 0.5 ng/ml. Bacterial lipopolysacharide (LPS) has been shown to be a potent inducer of procalcitonin release into systemic circulation. This release is not associated with an increase in calcitonin. Procalcitonin levels increase from 3 to 4 hours, peak at about 6 hours and then plateau for up to 24 hours. In contrast, C-reactive protein (CRP) levels rise between 12 and 18 hours after bacterial challenge. In blood serum, procalcitonin has a half-life of between 25 and 30 hours. A study showed that

hepatocytes produce large amounts of procalcitonin following stimulation with TNF-α and IL-6. In acute pancreatitis, procalcitonin closely correlates with the development of pancreatic infections.

4 TEST PRINCIPLE

In the Human Procalcitonin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human procalcitonin antibody. After 120 minutes incubation and washing, biotin labelled polyclonal anti-human procalcitonin antibody is added and incubated with captured procalcitonin for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of procalcitonin. A standard curve is constructed by plotting absorbance values versus procalcitonin concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5 PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling kit materials
- Do not drink, eat or smoke in the areas where kit materials are being handled
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6 TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7 REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.15 mL
Streptavidin HRP Conjugate	ready to use	13 mL
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	20 mL
Biotin-Ab Diluent	ready to use	13 mL
Wash Solution Conc. (10x)	concentrated	100 mL
Substrate Solution	ready to use	13 mL
Stop Solution	ready to use	13 mL
Instructions for Use + Certificate of Analysis	-	1 pc

8 MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10 - 1000 µL with disposable tips
- Multichannel pipette to deliver 100 µL with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550 - 650 nm)
- Software package facilitating data generation and analysis (optional)

9 PREPARATION OF REAGENTS

- **All reagents need to be brought to room temperature prior to use**
- **Always prepare only the appropriate quantity of reagents for your test**
- **Do not use components after the expiration date marked on their label**

9.1 Assay reagents supplied ready to use

Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2 °C - 8 °C and protected from the moisture.

Streptavidin-HRP Conjugate
Dilution Buffer
Biotin-Ab Diluent
Substrate Solution
Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2 °C - 8 °C.

9.2 Assay reagents supplied concentrated or lyophilized

Human Procalcitonin Master Standard:

Refer to Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

The resulting concentration of the human procalcitonin in the stock solution is **3200 pg/mL**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock		3200 pg/mL
250 µL of stock	250 µL	1600 pg/mL
250 µL of 1600 pg/mL	250 µL	800 pg/mL
250 µL of 800 pg/mL	250 µL	400 pg/mL
250 µL of 400 pg/mL	250 µL	200 pg/mL
250 µL of 200 pg/mL	250 µL	100 pg/mL
250 µL of 100 pg/mL	250 µL	50 pg/mL

Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Quality Controls.

Note:
Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with IFU and CoA and that ELISA test was carried out properly.

Biotin Labelled Antibody Conc. (100x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) with 99 parts Biotin-Ab Diluent.
Example:
10 µL of Biotin Labelled Antibody Concentrate (100x) + 990 µL of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Concentrate (100x) is stable 3 months when stored at 2 °C - 8 °C.
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Concentrate (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution.
Example: 100 mL of Wash Solution Concentrate (10x) + 900 mL of distilled water for use of all 96 wells.

Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2 °C - 8 °C. Opened Wash Solution Conc. (10x) is stable 3 months when stored at 2 °C - 8 °C.

10 PREPARATION OF SAMPLES

The kit measures human procalcitonin in serum and urine samples.

Samples should be assayed immediately after collection or should be stored at -20 °C.

Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results.
Avoid using hemolyzed or lipemic samples.

Serum samples:
Dilute samples **3x** with Dilution Buffer just prior to the assay,
e.g. 50 µL of sample + 100 µL of Dilution Buffer for singlets, or preferably 100 µL of sample + 200 µL of Dilution Buffer for duplicates.
Mix well (not to foam). Vortex is recommended.

Urine samples:
Dilute samples **2x** with Dilution Buffer just prior to the assay,
e.g. 75 µL of sample + 75 µL of Dilution Buffer for singlets, or preferably 150 µL of sample + 150 µL of Dilution Buffer for duplicates.
Mix well (not to foam). Vortex is recommended.

Stability and storage:
Serum samples should be stored at -20 °C, or preferably at -70 °C for long-term storage. Urine samples should be stored at -70 °C. Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

See Section on stability of samples when stored at 2 °C - 8 °C, effect of freezing/thawing on the concentration of procalcitonin.
Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

11 ASSAY PROCEDURE

1. Pipet **100 µL** of Standards, Quality Controls, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells.
   See Figure 1 for example of work sheet.
2. Incubate the plate at room temperature (ca. 25 °C) for **2 hours**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µL** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25 °C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.

7. Add **100 µL** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25 °C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µL** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature.
    The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20 °C.
    Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µL** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 -650 nm). Subtract readings at 630 nm (550-650 nm) from the readings at 450 nm.
    The absorbance should be read within 5 minutes following step 12.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine Procalcitonin concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.
Note 2: Manual washing: Aspirate wells and pipet 0.35 mL Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 3200	QC HIGH	Sample 7	Sample 15	Sample 23	Sample 31
B	Standard 1600	QC LOW	Sample 8	Sample 16	Sample 24	Sample 32
C	Standard 800	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	Standard 400	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	Standard 200	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	Standard 100	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	Standard 50	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

12 CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of procalcitonin (pg/mL) in samples.
Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.
The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 300 pg/mL (from standard curve) x 3 (dilution factor) = 900 pg/mL.

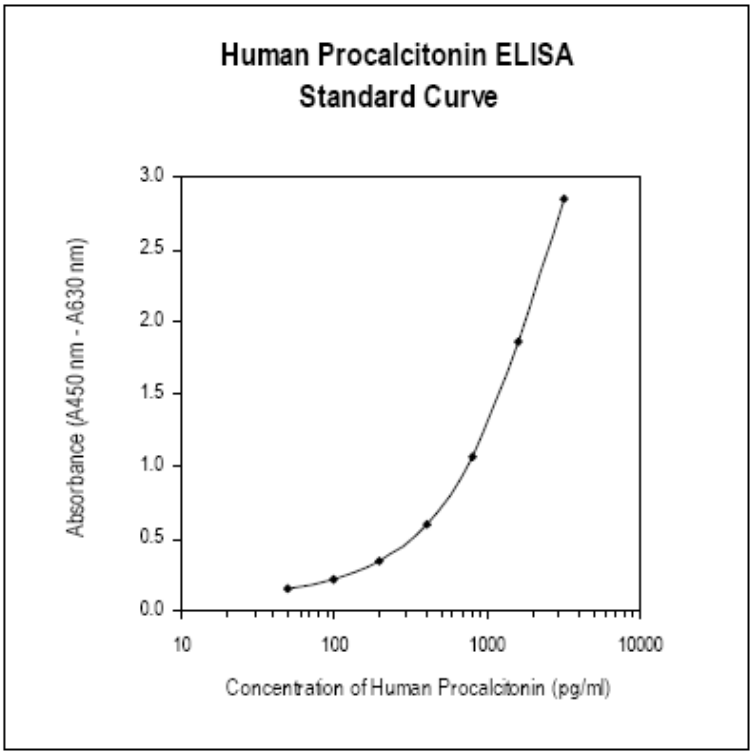

Figure 2: Typical Standard Curve for Human Procalcitonin ELISA.

13 Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological references ranges for procalcitonin levels with the assay.

14 DEFINITION OF THE STANDARD

The recombinant human procalcitonin is used as the Standard. The human procalcitonin (AA 1¬126), produced in E.coli, is 14 kDa protein containing 116 amino acid residues of the human procalcitonin and 10 AA extra.

15 METHOD COMPARISON

The Human Procalcitonin ELISA was compared to another commercial ECLIA immunoassay, by measuring 95 serum samples. The following correlation graph was obtained.

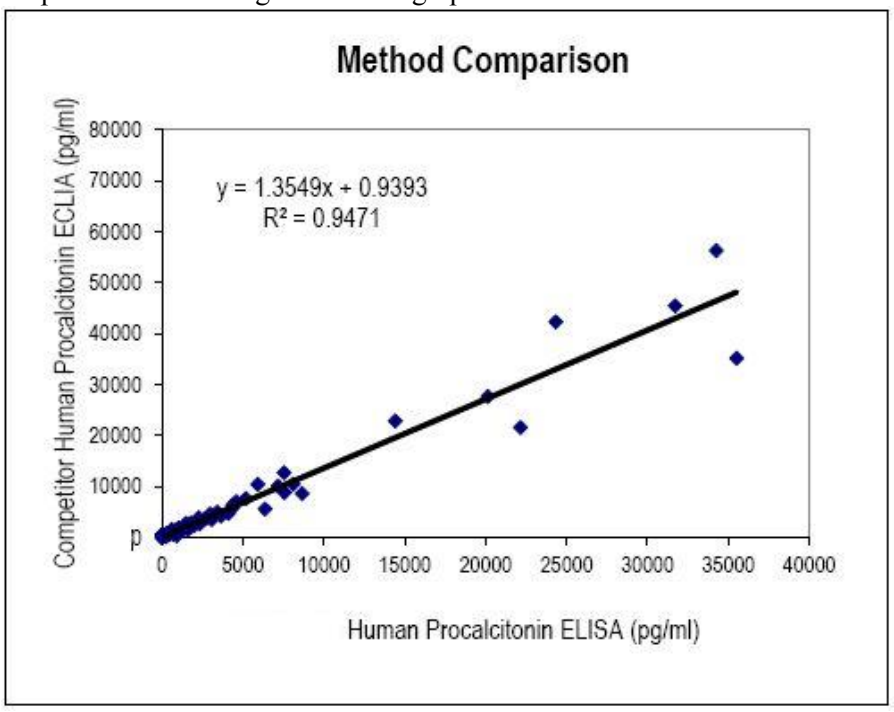

Figure 5: Method comparison.

16 TROUBLESHOOTING

Weak signal in all wells
Possible explanations:
- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells
Possible explanations:
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30 °C

High coefficient of variation (CV)
Possible explanation:
- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

17 REFERENCES

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