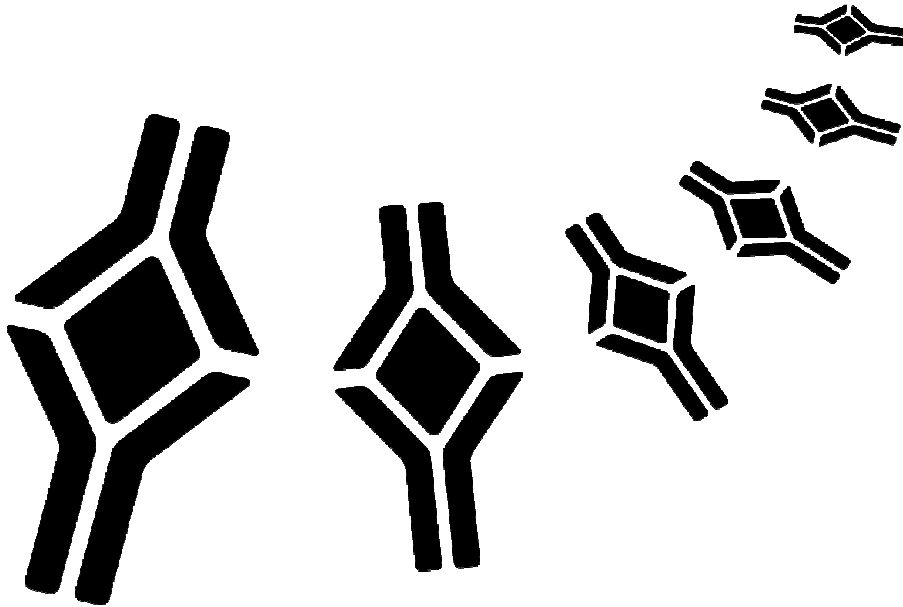


**BioVendor**

Research  
and Diagnostic Products



# HUMAN PROCALCITONIN ELISA

Product Data Sheet

Cat. No.: RD191006200R

For Research Use Only

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➤➤ **This kit is manufactured by:  
BioVendor – Laboratorní medicína a.s.**

➤➤ **Use only the current version of Product Data Sheet enclosed with the kit!**

## 1. INTENDED USE

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The RD191006200R Human Procalcitonin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human procalcitonin.

### »» Features

- **It is intended for research use only**
- The total assay time is less than 2.5 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

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

For stability of opened reagents see Chapter 9.

### 3. INTRODUCTION

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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 aminoprocaltitonin (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 lipopolysaccharide (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- $\alpha$  and IL-6. In acute pancreatitis, procalcitonin closely correlates with the development of pancreatic infections. Since procalcitonin has been reported to be increased in different non-septic conditions such as major trauma, acute respiratory distress syndrome, rejection after transplantation, cardiogenic shock, severe burns and heat-stroke, the discriminative power of procalcitonin could be hampered in these particular patient categories. A recent study concluded that children with bacterial pneumonia had significantly higher procalcitonin levels than those with a viral aetiology, but there was a significant degree of overlap. Procalcitonin has the greatest sensitivity and specificity for differentiating patients with SIRS from those with sepsis, when compared to IL-2, IL-6, IL-8, CRP and TNF- $\alpha$ . Today procalcitonin is considered to be one of the earliest and most specific markers of sepsis.

The antibodies used in this ELISA are specific for human Procalcitonin. Several quantitative procalcitonin assays are available, including a rapid, semiquantitative procalcitonin test. No assay detects the 116 kDa procalcitonin peptide exclusively. All assays detect various portions of several calcitonin precursors, using a combination of antibodies. Following the above, we can mention our procalcitonin crossreactivity (see Chapter 13. Specificity).

#### Areas of investigation:

Sepsis

Bacterial infection

Septic shock

Inflammation

## 4. TEST PRINCIPLE

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In the BioVendor Human Procalcitonin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human procalcitonin antibody. After 60 minutes incubation and washing, HRP labelled polyclonal anti-human procalcitonin antibody is added and incubated with captured procalcitonin for 60 minutes. After another 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 against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

## 5. PRECAUTIONS

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- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic 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

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- 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

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<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution Conc. (100x)	concentrated	0.13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	20 ml
Conjugate 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
Product Data Sheet + Certificate of Analysis	-	1 pc

## 8. MATERIAL REQUIRED BUT NOT SUPPLIED

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- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1000  $\mu\text{l}$  with disposable tips
- Multichannel pipette to deliver 100  $\mu\text{l}$  with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter 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  $450 \pm 10$  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

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- 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

- 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 2-8°C and protected from the moisture.

### **Dilution Buffer**

### **Conjugate Diluent**

### **Substrate Solution**

### **Stop Solution**

#### Stability and storage:

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

- **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:

<i>Volume of Standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
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 reconstituted Master Standard and/or 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 PDS and CoA and that ELISA test was carried out properly.*

**Conjugate Solution Conc. (100x)**

Prepare the working Conjugate Solution by adding 1 part Conjugate Solution Concentrate (100x) with 99 parts Conjugate Diluent. Example: 10 µl of Conjugate Solution Concentrate (100x) + 990 µl of Conjugate Diluent for 1 strip (8 wells).

Stability and storage:

Opened Conjugate Solution Concentrate (100x) is stable 3 months when stored at 2-8°C.

**Do not store the diluted Conjugate Solution.**



### **Wash Solution Conc. (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 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

## 10. PREPARATION OF SAMPLES

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The kit measures human procalcitonin in serum and urine samples.

Samples should be assayed immediately after collection or should be stored at -20°C or -70°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 Chapter 13 for stability of samples when stored at 2-8°C and 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

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1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and 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 **1 hour**, 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 Conjugate 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 Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. 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.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. 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 9.**

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

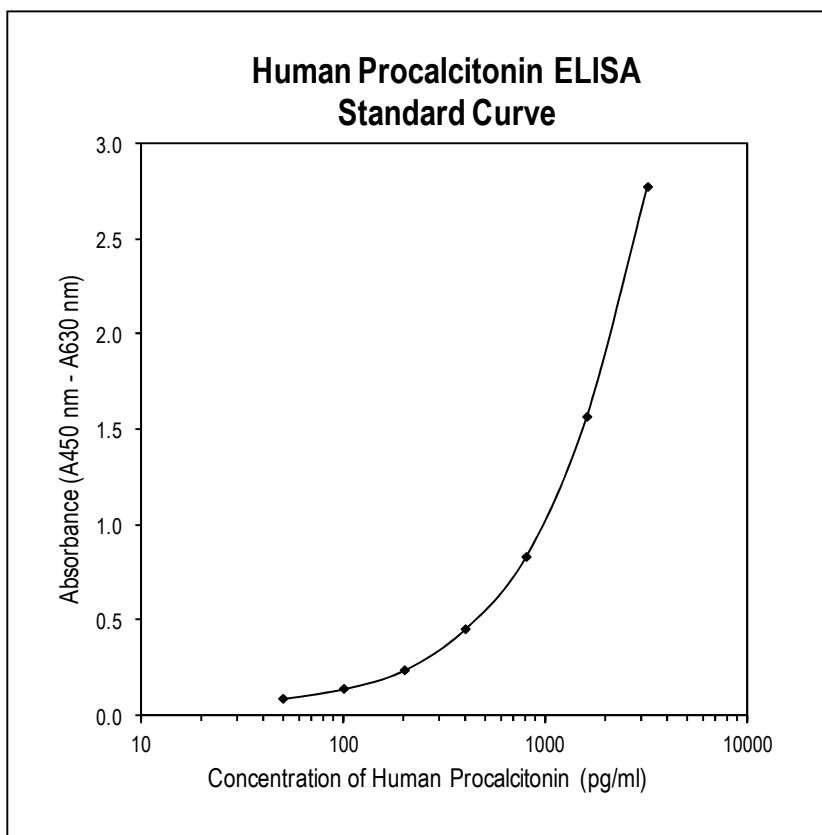
Figure 1: Example of a work sheet.

## 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.*

## PERFORMANCE CHARACTERISTICS

➤➤ Typical analytical data of BioVendor Human Procalcitonin ELISA are presented in this chapter

- **Sensitivity**

Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank:  $(A_{\text{blank}} + xSD_{\text{blank}})$ ) is calculated from the real human procalcitonin values in wells and is 15 pg/ml.

\* Dilution Buffer is pipetted into Blank wells.

- **Limit of assay**

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**

The antibodies used in this ELISA detect human katalcalcin with 20% crossreactivity, human aminoprocaltitonin with 50% crossreactivity and there is no detectable crossreactivity with human calcitonin.

Sera of several mammalian species were measured in the assay. See results below.

For details please contact us at [info@biovendor.com](mailto:info@biovendor.com)

<i>Mammalian serum sample</i>	<i>Observed crossreactivity</i>
Bovine	no
Cat	no
Dog	yes
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	yes

➤➤ Presented results are multiplied by respective dilution factor

• **Precision**

Intra-assay (Within-Run) (n=8)

<i>Sample</i>	<i>Mean (pg/ml)</i>	<i>SD (pg/ml)</i>	<i>CV (%)</i>
1	3 302.6	101.8	3.1
2	2 168.9	48.2	2.2

Inter-assay (Run-to-Run) (n=6)

<i>Sample</i>	<i>Mean (pg/ml)</i>	<i>SD (pg/ml)</i>	<i>CV (%)</i>
1	1 523.9	96.8	6.4
2	2 325.6	73.0	3.1

• **Spiking Recovery**

Serum samples were spiked with different amounts of human Procalcitonin and assayed.

<i>Sample</i>	<i>Observed (pg/ml)</i>	<i>Expected (pg/ml)</i>	<i>Recovery O/E (%)</i>
1	752.5	-	-
	3 281.9	3 152.5	104.1
	2 011.9	1 952.5	103.0
	1 375.3	1 352.5	101.7
2	2 217.3	-	-
	7 349.1	7 017.3	104.7
	4 563.1	4 617.3	98.8
	3 371.1	3 417.3	98.6

• **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (pg/ml)</i>	<i>Expected (pg/ml)</i>	<i>Recovery O/E (%)</i>
1	-	3 465.7	-	-
	2x	1 639.5	1 732.9	94.6
	4x	846.9	866.4	97.7
	8x	430.2	433.2	99.3
2	-	2 558.9	-	-
	2x	1 225.3	1 279.5	95.8
	4x	641.8	639.6	100.3
	8x	332.5	319.8	103.9

- **Stability of samples stored at 2-8°C**

Serum samples should be stored at -20°C. However, no significant decline in concentration of human procalcitonin was observed in serum samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.05%, respectively.

<i>Sample</i>	<i>Temp, incubation, period</i>	<i>Serum (pg/ml)</i>
1	-20°C	1193.0
	2-8°C, 1 day	1210.1
	2-8°C, 7 days	1086.2
2	-20°C	1259.9
	2-8°C, 1 day	1283.0
	2-8°C, 7 days	1377.8
3	-20°C	806.2
	2-8°C, 1 day	783.6
	2-8°C, 7 days	741.3

- **Effect of Freezing/Thawing**

No significant decline was observed in concentration of human procalcitonin in serum and urine samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

<i>Sample</i>	<i>Number of f/t cycles</i>	<i>Serum (pg/ml)</i>	<i>Urine (pg/ml)</i>
1	1x	2381.2	1226.8
	3x	2479.9	1162.7
	5x	2475.3	1240.5
2	1x	1054.2	818.5
	3x	1029.7	842.8
	5x	1091.6	780.1
3	1x	809.0	746.0
	3x	869.1	745.2
	5x	834.7	719.6

- **Reference range**

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.

### 13. DEFINITION OF THE STANDARD

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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 extra AA.

### 14. METHOD COMPARISON

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The Biovendor Human Procalcitonin ELISA was compared to another commercial ECLIA immunoassay, by measuring 67 serum samples. The following correlation graph was obtained.

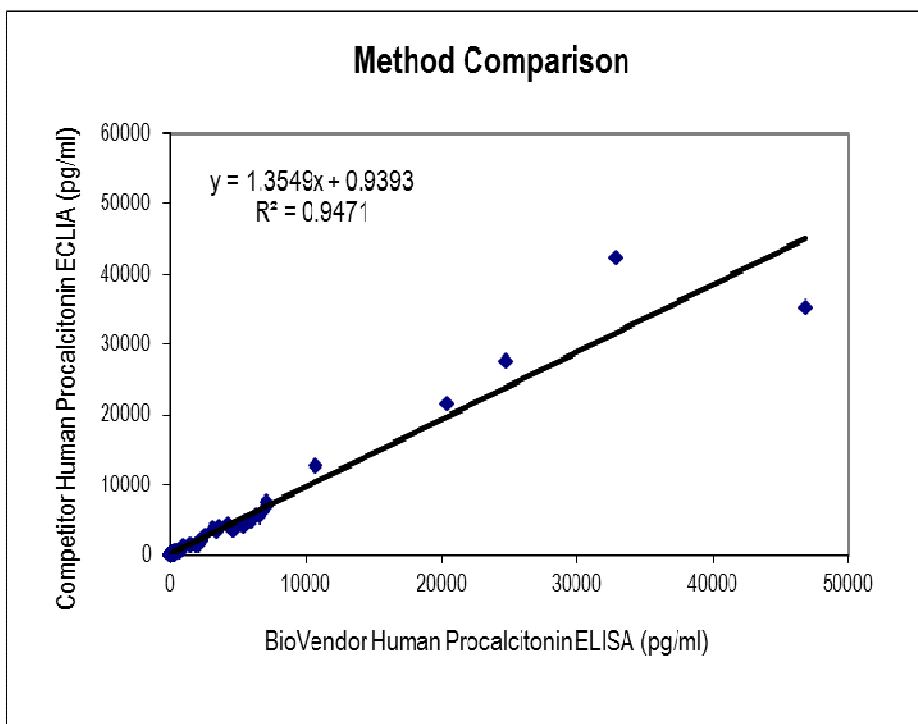


Figure 3: Method Comparison.



## 15. TROUBLESHOOTING AND FAQs

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### »» 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







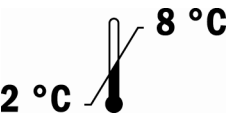

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### »» References to human procalcitonin

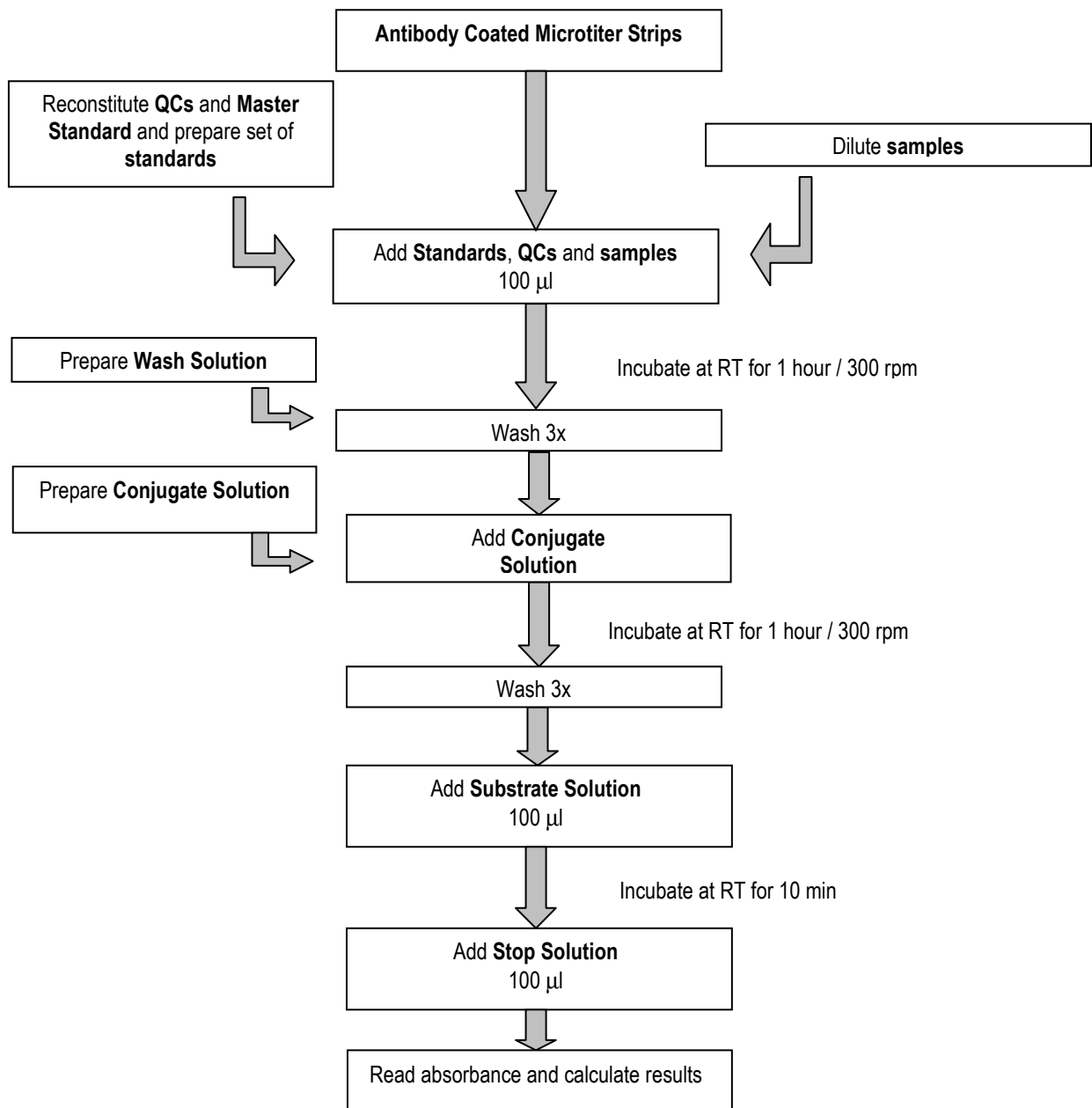
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»» For more references on this product see our WebPages at [www.biovendor.com](http://www.biovendor.com)

## 18. EXPLANATION OF SYMBOLS

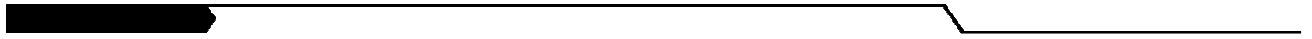
	Catalogue number
	Content
	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

## Assay Procedure Summary





## NOTES

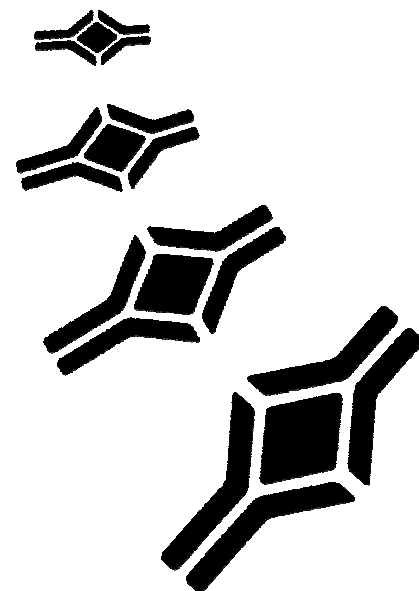


**NOTES**



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Research  
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