

# Human Intact Proinsulin ELISA

Cat. No. RD193094100



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Use only the actual version of Product Data Sheet enclosed with the kit!

## 1. Intended Use

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The RD193094100 Human Intact Proinsulin ELISA is a sensitive two-site sandwich enzyme immunoassay for the quantitative measurement of human intact proinsulin in serum. It is intended for *in vitro* diagnostic use.

### Features

- The total assay time is less than two hours.
- The kit measures total intact human proinsulin.
- Calibrators are human serum based.
- No dilution of serum samples.
- Components of the kit are in the lyophilized, concentrated and ready-to-use states.
- Convenient for automatization.

## 2. Storage, Expiration

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

### 3. Summary

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Proinsulin (PI), a polypeptide of 9390 MW (86 amino acids) is synthesized in the  $\beta$  cells of the Islets of Langerhans in the pancreas. The sequence of proinsulin is highly conserved in mammalian species and is homologous with IGF-1 and IGF-2.

This protein is processed to C-peptide and insulin forms. Both are secreted in equimolar amounts into the blood. In normal individuals, proinsulin is present in the circulation in very low concentrations (typical basal values 2-6 pmol/l). The level of proinsulin in serum can be a reflection of  $\beta$  cell status and a consequence of dysfunction of PI processing and/or secretion. Proinsulin like material is increased in clinical conditions as insulinoma, familial hyperinsulinemia, non-insulin dependent diabetes mellitus.

#### Clinical applications

Proinsulin is the precursor of insulin, which is the principal hormone responsible for the control of glucose metabolism. The measurement of Proinsulin in serum provides useful valuable information for the diagnosis of insulinomas. Proinsulin levels have also been shown to be elevated in non-insulin dependent diabetics (NIDDM), in insulin dependent diabetics (IDDM) and other clinical situations.

### 4. Test Principle

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Prior to use a Blocking Buffer is added to the allocated wells in the BioVendor Human Intact Proinsulin ELISA. Then, Calibrators, Quality Controls and samples of sera are incubated in microtitration wells coated with a monoclonal anti-human proinsulin antibody (S2) specific for the epitope at the C-peptide/insuline A chain junction. S2 able to bind intact PI, des (31,32)-PI and split (32,33)-PI but not insulin, C-peptide and the other "des" and "split" forms. Any human proinsulin present is captured by immobilized antibody and unbound protein is washed away after the first incubation period. Then, a horseradish peroxidase (HRP) labelled anti-human proinsulin antibody (S53) is added into the wells and incubated with proinsulin. This antibody is specific for the epitopes at insulin  $\beta$  chain/C-peptide junction. S53 is able to bind to intact PI, des (64, 65)-PI but not insulin, C-peptide and others "des" and "split" form. Following another washing step, to remove unbound antibody-HRP conjugate, the substrate solution is added to the wells. The enzymatic reaction yields a blue product that turns yellow when acidic Stop Solution is added. Absorbance of the resulting yellow colour product is measured at 450 nm. The intensity of colour development is proportional to the concentration of human proinsulin. A standard curve is constructed by plotting absorbance values against concentrations of proinsulin calibrators using a four-parameter function, and concentrations of unknown samples are determined using this standard curve.

**The combination of the two MAbs has the ability to detect only the intact human proinsulin.**

## 5. Precautions

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- For *in vitro* diagnostic use.
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, for HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no tests can guarantee the complete absence of infectious agents.
- Avoid contact with the acidic Stop Solution, and Substrate Solution, which contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. Substrate Solution and/or Stop Solution 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.
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- Use thoroughly clean glassware.
- Use distilled water, stored in clean containers.
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution 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 right away addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Reagents with different lot numbers or sources should not be mixed.
- Do not use kit reagents after the expiration date indicated on the box label.

## 6. Reagents Supplied

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<i>Cat. No.</i>	<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
C391211	Antibody Coated Microtiter Strips	ready to use	96 wells
C392211	Conjugate Solution	ready to use	11 ml
C393151	Proinsulin 0-5 Calibrators	lyophilized	6 x 1 vial
C394151	Quality Control High	lyophilized	1 vial
C394251	Quality Control Low	lyophilized	1 vial
C399111	Blocking Buffer	ready to use	1.5 ml
C396021	Wash Solution (10x)	concentrated	40 ml
C397111	Substrate Solution	ready to use	15 ml
C398121	Stop Solution	ready to use	15 ml
-	Instruction Manual + Certificate of Analysis		1 pc

## 7. Materials Required but Not Supplied

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- Precision pipettes to deliver 10-1000  $\mu$ l and disposable tips
- Multichannel pipette 50-200  $\mu$ l and disposable tips
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water
- Orbital microplate shaker capable of approximately 150 rpm (optional)
- Microplate washer (optional). Manual washing is possible but not preferable
- Microplate reader with 450 and 650  $\pm$  10 nm filter
- Software package facilitating data generation and analysis
- Absorbent material e.g. paper towels for blotting the microtiter plate after washing

## **8. Preparation of Reagents**

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All reagents need to be brought to room temperature prior to the assay.

Assay reagents are supplied ready-to-use, with the exception of Proinsulin 0 Calibrator, Proinsulin 1-5 Calibrators, Quality Control High and Low and Wash Solution Concentrated (10x).

The kit may be used for sequential utilization. When preparing working strength solution, use only the amount of reagents needed for the analysis.

- If you do not use the whole plate, return unused strips in the provided aluminium bag with dessicant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

### Preparation of reagents for 1 plate:

#### **Wash Solution:**

Quantitatively dilute 40 ml of Wash Solution Concentrate (10x) with 360 ml of deionized (distilled) water to the final total volume 400 ml of Wash Solution (1x) and mix thoroughly.

#### Stability and storage:

The diluted Wash Solution is stable for 6 months if stored at 2-8°C.

#### **Proinsulin 0 Calibrator:**

Reconstitute Proinsulin 0 Calibrator with 3.0 ml of deionized (distilled) water. Mix gently the reconstituted Calibrator 0 and allow it to sit for about 10 minutes optimally (to ensure complete reconstitution). Avoid foaming when reconstituting or mixing the protein solutions.

#### Stability and storage:

Reconstituted Calibrator should be frozen at -20°C until next use. Avoid multiple freeze-thaw cycles (max. 2 times). Reconstituted Calibrator is stable until the expiration date (see label on the vial) if stored under this condition.

#### **Proinsulin 1-5 Calibrators:**

Reconstitute Proinsulin 1-5 Calibrators with 1.0 ml of deionized (distilled) water. Mix gently reconstituted Calibrators and allow them to sit for about 10 minutes optimally (to ensure complete reconstitution). Avoid foaming when reconstituting or mixing the protein solutions.

Stability and storage:

Reconstituted Calibrators should be frozen at -20°C until next use. Avoid multiple freeze-thaw cycles (max. 2 times). Reconstituted Calibrators are stable until the expiration date (see label on the vial) if stored under this condition.

**Quality Controls, High, Low:**

Refer to the Certificate of Analysis for actual Quality Controls values.

Reconstitute Quality controls with 1.0 ml of deionized (distilled) water. Mix gently the reconstituted Quality Controls and allow them to sit for about 10 minutes optimally (to ensure complete reconstitution). Avoid foaming when reconstituting or mixing the protein solutions.

Thus reconstituted Quality Controls are ready to use.

Stability and storage:

Reconstituted Quality Controls are stable until the expiration date (see label on the vial) if stored at -20°C. Avoid multiple freeze-thaw cycles (max. 2 times).

## **9. Preparation of Samples**

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It is recommended to use serum samples. Avoid using hemolyzed, lipemic or bacterially contaminated sera.

**The samples are not diluted!**

Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze-thaw cycles, which may cause loss of antibody activity and give erroneous results.

Stability and storage:

Samples may be stored at 2-8°C for up to 24 hours. For long-term storage, samples should be stored frozen at -20°C, or preferably at -70°C.

*Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results!*

## 10. Assay Procedure

- 1) Prepare reagents as directed in the previous sections. Remove excess microplate strips from the plate frame.
- 2) Allocate the wells of Microtiter Plate for calibrators, control sera and samples. See Figure 1 for example of work sheet.
- 3) Pipette 10  $\mu$ l of Blocking Buffer directly into the bottom of the wells **except blanks**.
- 4) Pipette 100  $\mu$ l of reconstituted Calibrators (0-5), Quality controls and samples, preferably in duplicates, into the appropriate wells. Leave the G1, G2 wells empty (=Blank).
- 5) Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at ca. 150 rpm on an orbital microplate shaker. (The incubation time begins after the last sample addition).
- 6) Wash the wells 3-times with diluted Wash Solution (0.35 ml per well). Aspirate all liquid from the wells. Invert the plate and blot it against a clean paper towels to remove the remaining Washing Buffer.
- 7) Add 100  $\mu$ l of Conjugate Solution into each well except Blank.
- 8) Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 150 rpm on an orbital microplate shaker.
- 9) Wash the wells 3-times with Wash Solution (0.35 ml per well) and blot it.
- 10) Add 200  $\mu$ l of Substrate Solution into each well. Avoid exposing the Microtiter Plate to direct light. (Covering the plate with e.g. aluminium foil is recommended.)
- 11) Incubate the plate at room temperature for 15 minutes, shaking at ca. 150 rpm on an orbital microplate shaker. (The incubation time begins after the first TMB addition).
- 12) Stop the colour development by adding 100  $\mu$ l of Stop Solution into each well.
- 13) Determine the absorbance by reading the plate at 450, 405 and 620 nm (as a reference). The absorbance must be read within 5-15 minutes following step 12.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Calibrator 5	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
B	Calibrator 4	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Calibrator 3	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Calibrator 2	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Calibrator 1	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Calibrator 0	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	QC High	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of work sheet.

## 11. Calculations

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In order to obtain a better sensitivity, the present method employs spectrophotometric reading at two wavelengths (450 and 405 nm). For all O.D. overflow at 450 nm, multiplied the O.D. 405 nm by the correction factor calculated by the ratio between O.D. 450 nm and O.D. 405 nm. To establish this factor, used a sample reading at 405 and 450 nm.

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance at 450 nm (Y) of Calibrators against *log* of the known concentration (X) of Calibrators, using the four-parameter algorithm. Results are reported as concentration of proinsulin (pmol/l) in samples.

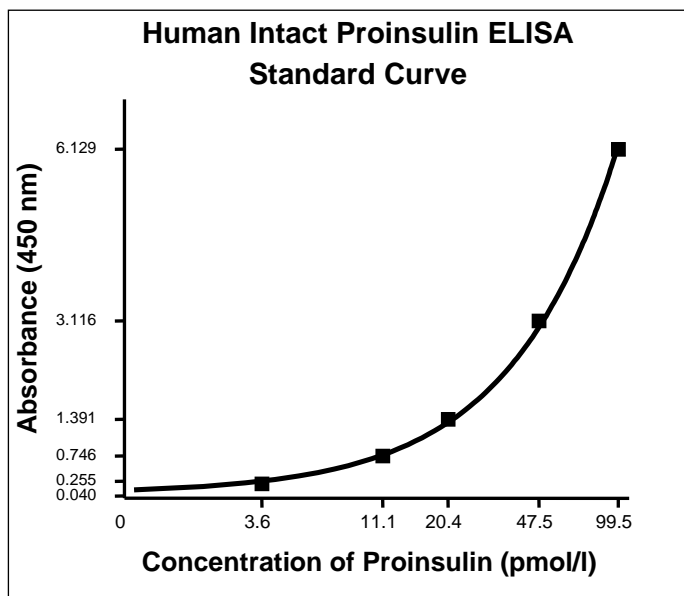


Figure 2: Typical Standard Curve for Proinsulin

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 Calibrators).

## 12. Performance Characteristics

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Typical analytical data obtained with the BioVendor Human Intact Proinsulin ELISA are presented in this chapter. **For actual Standard curve and Quality Controls values see the Certificate of Analysis.**

- **Sensitivity**

The limit of detection (defined as human proinsulin concentration giving absorbance higher than mean absorbance of blank plus two standard deviations of the absorbance of blank:  $A_{\text{blank}} + 2xSD_{\text{blank}}$ ) is defined as follows:

Analytical Limit of Detection is calculated from the real Proinsulin values in wells and is 0.60 pmol/l

Assay Sensitivity takes the dilution of samples into consideration and is calculated according to the formula: Assay Sensitivity = Analytical Limit of Detection x sample dilution = 0.60 pmol/l x 1 = 0.60 pmol/l

- **Specificity**

Patient samples may contain human anti-mouse antibodies (HAMA), which are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies. This assay has been designed to minimize interference from HAMA-containing specimens with the use of blocking HAMA interferences (Blocking Buffer). Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.

- **Cross-reactivity**

The antibodies used in the Human Intact Proinsulin ELISA kit are highly specific to human intact proinsulin.

The following peptides were tested and no cross-reactivity has been observed:

Human Insulin:	< 10 0000 pmol/l
Human C-Peptide:	50 0000 pmol/l
Des (31,32)-Proinsulin:	< 200 pmol/l
Split (32,33)-Proinsulin:	5 000 pmol/l
Des (64,65) -Proinsulin:	200 pmol/l
Split (65,66) -Proinsulin:	1 000 pmol/l

No crossreactivity to the following animal sera was found:  
mouse, rat, bovine, goat, sheep, pig, dog, monkey and hamster.

- Precision

Intra-assay (Within-Run) (n=20)

<i>Sample</i>	<i>Mean (pmol/l)</i>	<i>Standard Deviation</i>	<i>CV (%)</i>
1	9.4	0.3	3.2
2	23.7	0.7	3.0
3	41.9	1.2	2.9

Inter-assay (Run-to-Run) (n=9)

<i>Sample</i>	<i>Mean (pmol/l)</i>	<i>Standard Deviation</i>	<i>CV (%)</i>
1	7.9	0.2	2.5
2	14.1	0.2	1.4
3	29.1	0.4	1.4

- Spiking Recovery

Serum sample was spiked with Calibrators 0-5 and assayed.

<i>Sample</i>	<i>Observed (pmol/l)</i>	<i>Expected (pmol/l)</i>	<i>Recovery O/E (%)</i>
S	8.7	-	-
S+Cal 0	4.5	4.3	104.7
S+Cal 1	6.0	6.1	98.4
S+Cal 2	10.1	9.9	102.0
S+Cal 3	15.1	14.5	104.1
S+Cal 4	28.7	28.1	102.1
S+Cal 5	56.9	54.1	105.2

- **Dilution Linearity**

Serum samples were further serially diluted with Dilution Buffer (see table below) and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (pmol/l)</i>	<i>Expected (pmol/l)</i>	<i>Recovery O/E (%)</i>
1	-	22.5	-	-
	1:2	11.8	11.2	105
	1:4	6.4	5.6	114
	1:8	4.0	3.7	107
	1:10	2.8	2.8	110
	1:20	2.0	2.2	90

- **Normal Values**

Determined normal values are only indicative since they may be affected by various agents. We recommend to establish it is own normal range for each laboratory.

<i>Sample</i>	<i>Number of samples</i>	<i>Mean ± SD (pmol/l)</i>
Non obese fasting patients	16	2.56 ± 1.28
Fasting IDDM	9	4.67 ± 3.85
Non-fasting IDDM	13	16.25 ± 17.75

### 13. Method Comparison

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The BioVendor Human Intact Proinsulin ELISA was compared to commercial immunoassay (ELISA, supplier: A), measuring of 72 serum samples, with the following results:

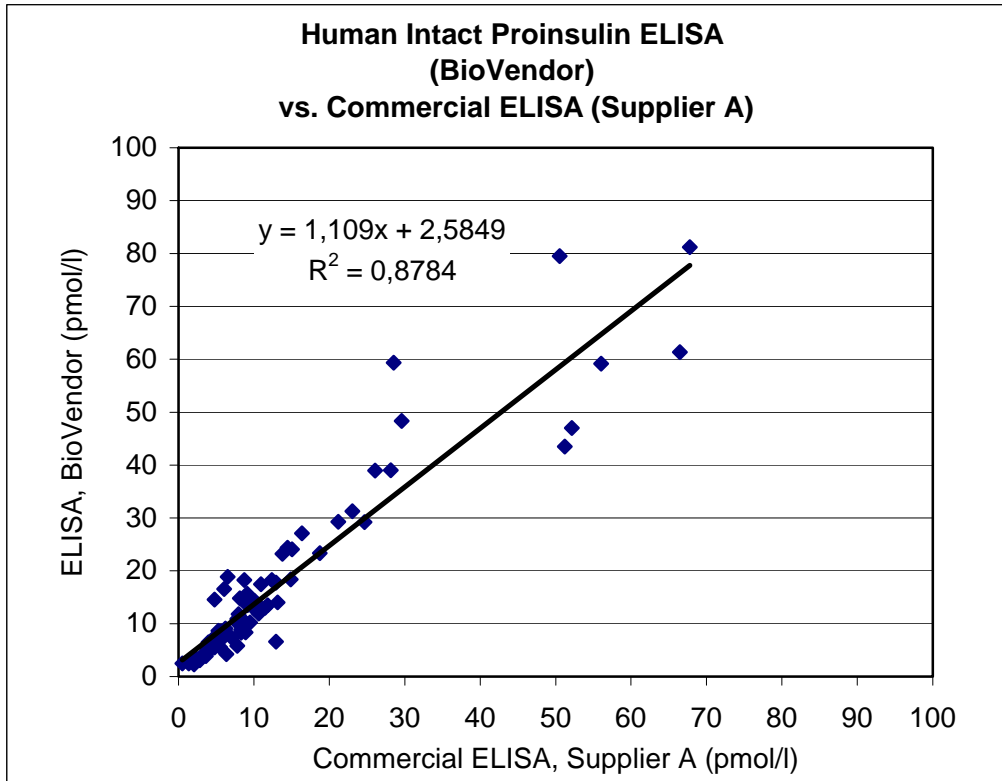


Figure 3: Method comparison

## 14. Troubleshooting and FAQs

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### 1/ 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 and samples were allowed to warm up at room temperature
- Inadequate laboratory temperature
- Reagents exposure to excessive temperature

### 2/ High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time should be decreased before addition of Stop Solution
- Inadequate laboratory temperature
- Reagents e.g. Substrate Solution is contaminated with oxidative active substances

### 3/ High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing and aspiration of fluid
- Non-homogenous sample after thawing
- Insufficient mixing of reagents and samples before use
- Hemolytic or chylous sera, turbidity, particles or high lipid content of the sample
- Carry over between samples/calibrators and/or using of the same tip to pipette different reagents
- Unequal volumes added to the wells

### 4/ Drift

- Inadequate rpm of orbital shaker
- Inadequate rehydration volume for the calibrators
- Reagents exposure to bacterial contamination
- Expiration date for the reagent exceeded

## 15. References

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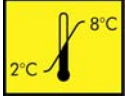


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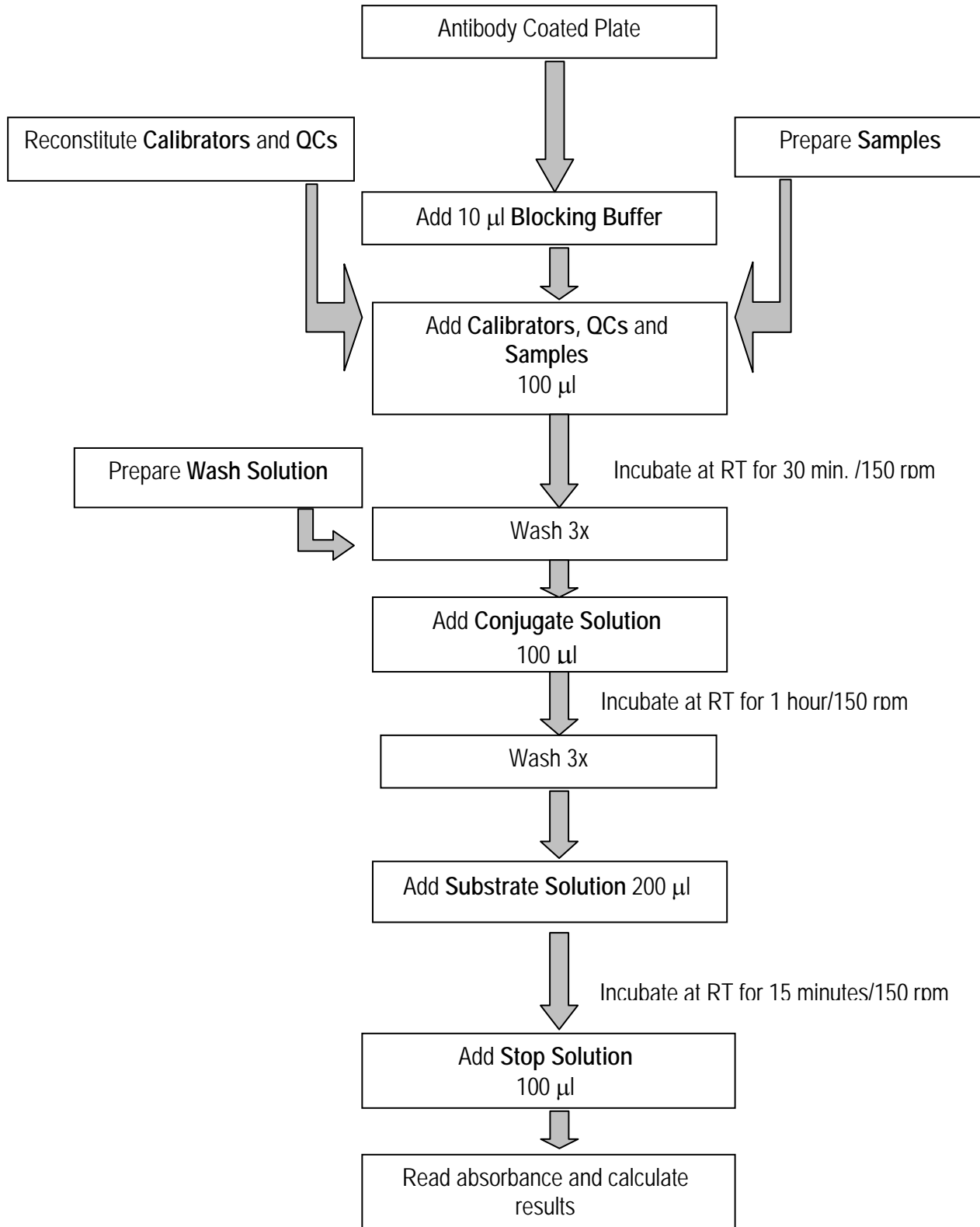
For more references on this product  
see our WebPages at [www.biovendor.com](http://www.biovendor.com)

## 16. Explanation of Symbols

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	<u>Catalogue number</u>
	<u>Content</u>
	<u>Lot number</u>
	<u>See instructions for use</u>
	<u>Biological hazard</u>
	<u>Expiry date</u>
	<u>Storage conditions</u>
	<u>Identification of packaging materials</u>
	<u>In vitro diagnostic medical device</u>

### Assay Procedure Summary



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2								
1								
	A	B	C	D	E	F	G	H

Notes:

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