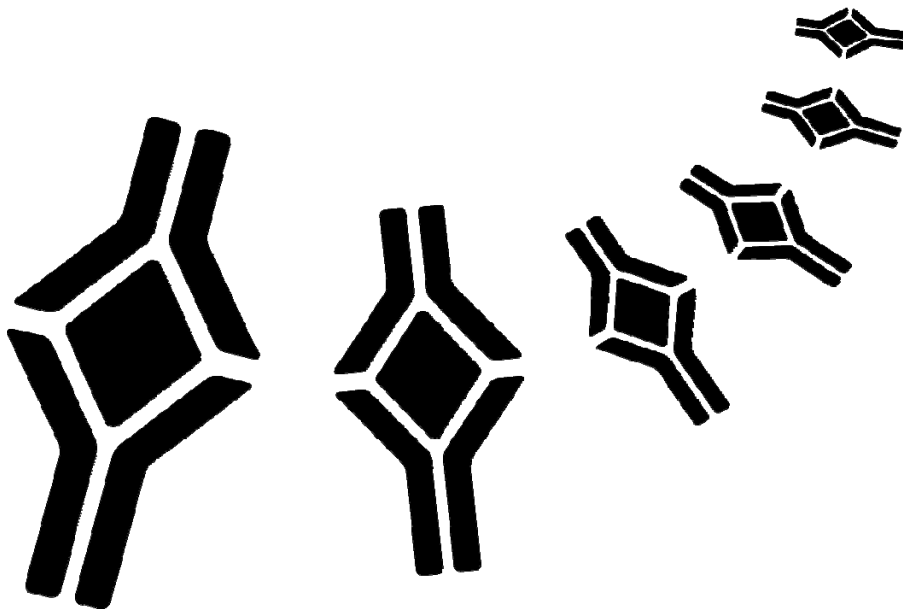


BioVendor

Research
and Diagnostic Products



HUMAN INTACT PROINSULIN ELISA

Product Data Sheet

Cat. No.: RD193094100

European
Union:



Rest of the world:
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

The RD193094100 Human Intact Proinsulin ELISA is a sensitive two-site sandwich enzyme immunoassay for the quantitative measurement of human intact proinsulin.

»» Features

- **European Union: for *in vitro* diagnostic use.**
Rest of the world: for research use only!
- The total assay time is less than two hours.
- The kit measures total intact human proinsulin in serum.
- 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

Store the 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

Proinsulin (PI), a polypeptide of 9390 MW (86 amino acids) is synthesized in the β 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 β 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.

Area of investigation:

Diabetology

4. TEST PRINCIPLE

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 β 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. 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

- **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, 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.
- 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

| <i>Kit Components</i> | <i>State</i> | <i>Quantity</i> |
|--|--------------|-----------------|
| Antibody Coated Microtiter Strips | ready to use | 96 wells |
| Conjugate Solution | ready to use | 11 ml |
| Proinsulin 0-5 Calibrators | lyophilized | 6 x 1 vial |
| Quality Control HIGH | lyophilized | 1 vial |
| Quality Control LOW | lyophilized | 1 vial |
| Blocking Buffer | ready to use | 1.5 ml |
| Wash Solution (10x) | concentrated | 40 ml |
| Substrate Solution | ready to use | 2 x 13 ml |
| Stop Solution | ready to use | 15 ml |
| Product Data Sheet + Certificate of Analysis | | 1 pc |

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Glassware (graduated cylinder and bottle) for Wash Solution
- Precision pipettes to deliver 10-1000 μ l with disposable tips
- Multichannel pipette to deliver 50-200 μ l with disposable tips
- Orbital microplate shaker capable of approximately 150 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Microplate reader with 405 and 450 nm filters, 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.
- 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.

Blocking Buffer

Conjugate Solution

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:

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

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).

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.

10. PREPARATION OF SAMPLES

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!

11. 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 µl** of Blocking Buffer directly into the bottom of the wells **except blanks**.
- 4) Pipette **100 µl** 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 µl** 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 µl** 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 µl** of Stop Solution into each well.
- 13) Determine the absorbance by reading the plate at 450, 405 and 630 nm (as a reference). The absorbance must be read within 20 minutes following step 12.

Note: 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 | 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 a work sheet.

12. CALCULATIONS

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 (Y) of Calibrators against the known concentration (X) of Calibrators in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of proinsulin (pmol/l) in samples.

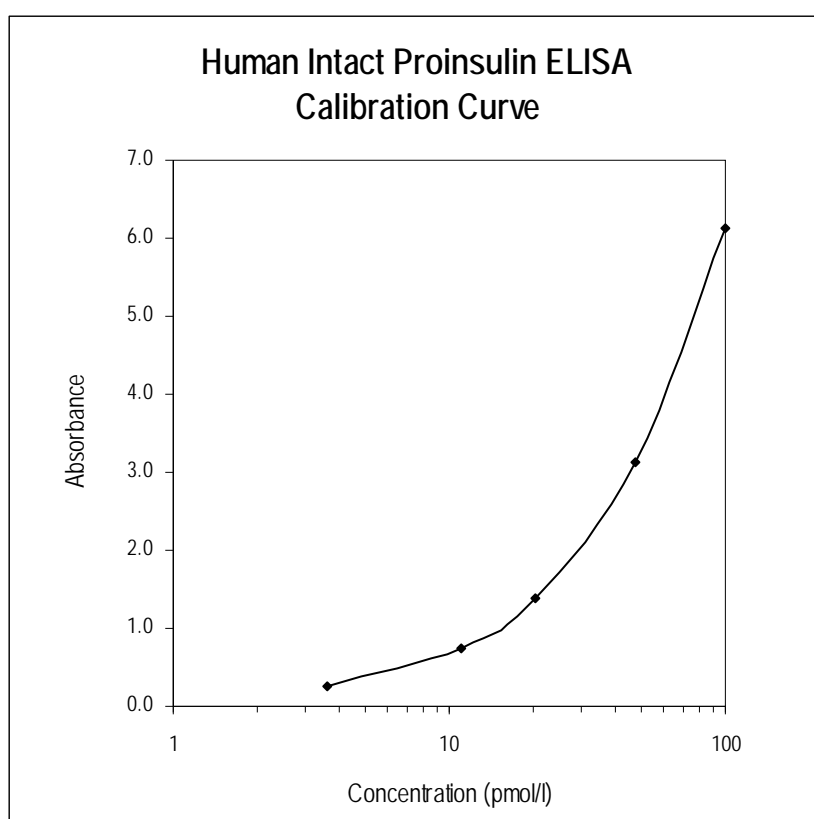


Figure 2: Typical Calibration Curve for Human Intact Proinsulin ELISA

13. PERFORMANCE CHARACTERISTICS

»» Typical analytical data of BioVendor Human Intact Proinsulin 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 two standard deviations of the absorbance of blank: $A_{\text{blank}} + 2 \times \text{SD}_{\text{blank}}$, is calculated from the real human proinsulin values in wells and is 0.60 pmol/l.

*Dilution Buffer is pipetted into blank wells.

- **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: | < 10 000 pmol/l |
| Human C-Peptide: | 50 000 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 |

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

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

- 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 | - | - |
| | 2x | 11.8 | 11.2 | 105 |
| | 4x | 6.4 | 5.6 | 114 |
| | 8x | 4.0 | 3.7 | 107 |
| | 10x | 2.8 | 2.8 | 110 |
| | 20x | 2.0 | 2.2 | 90 |

- **Normal Values**

Determined normal values are only indicative since they may be affected by various agents. We recommend that each laboratory establish its own normal range.

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

14. METHOD COMPARISON

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

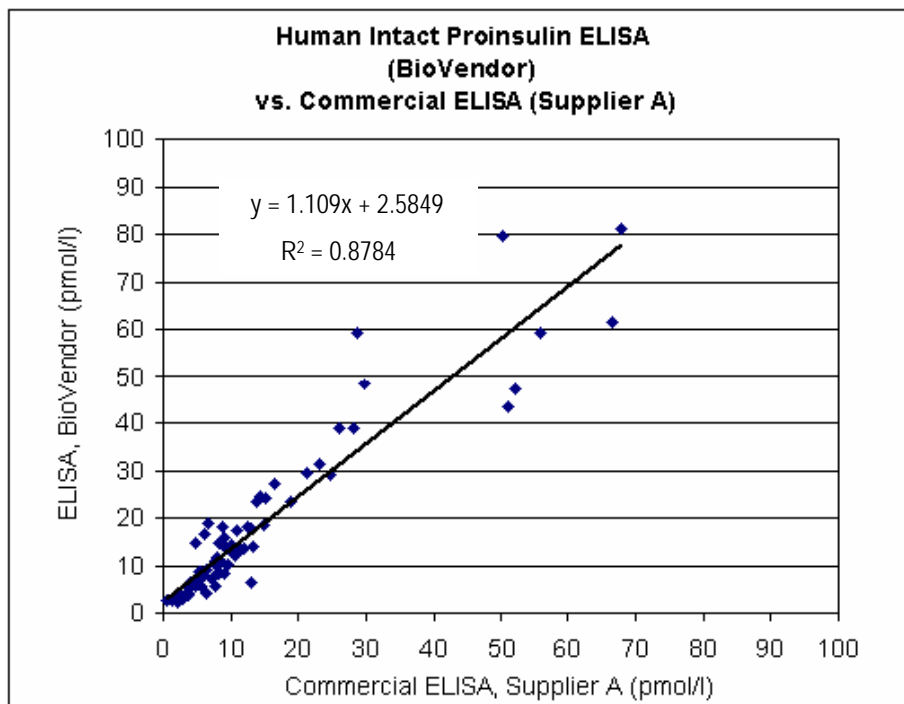


Figure 3: Method comparison

15. TROUBLESHOOTING AND FAQs

»» 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

»» 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

»» 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

»» Drift

Possible explanation:

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







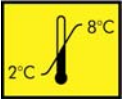


16. REFERENCES

»» References to Proinsulin:

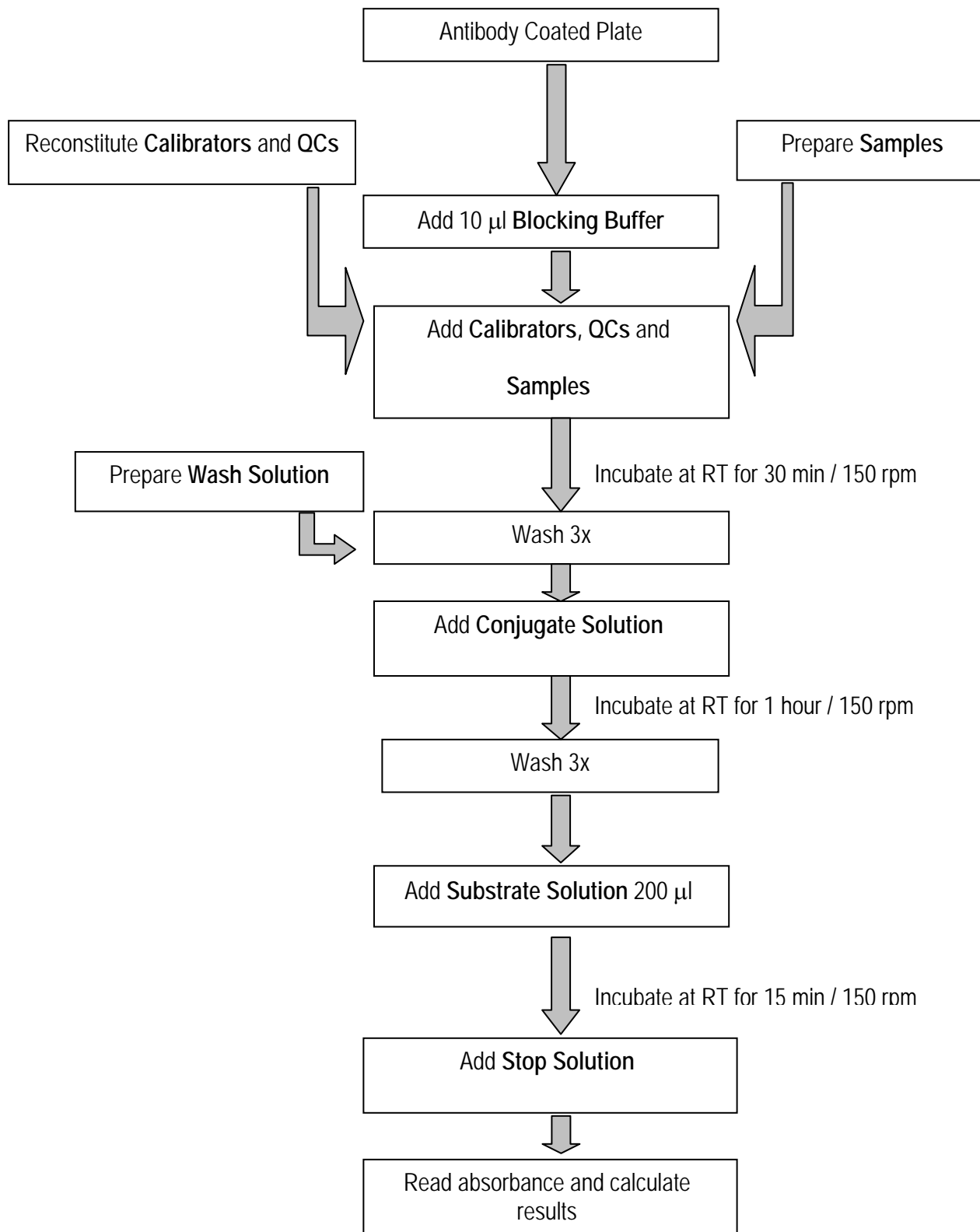
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- Kricka L.: Human anti-animal antibody interferences in immunological assays. *Clinical Chemistry* (1999); **45**: 942-956

»» For more references on this product see our WebPages at www.biovendor.com

17. EXPLANATION OF SYMBOLS

| | |
|---|---------------------------------------|
|  | Catalogue number |
|  | Content |
|  | Lot number |
|  | See instructions for use |
|  | Biological hazard |
|  | Expiry date |
|  | Storage conditions |
|  | Identification of packaging materials |
|  | In vitro diagnostic medical device |

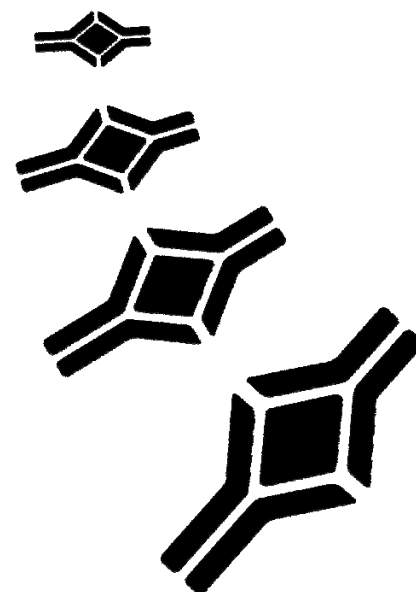
Assay Procedure Summary



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NOTES





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