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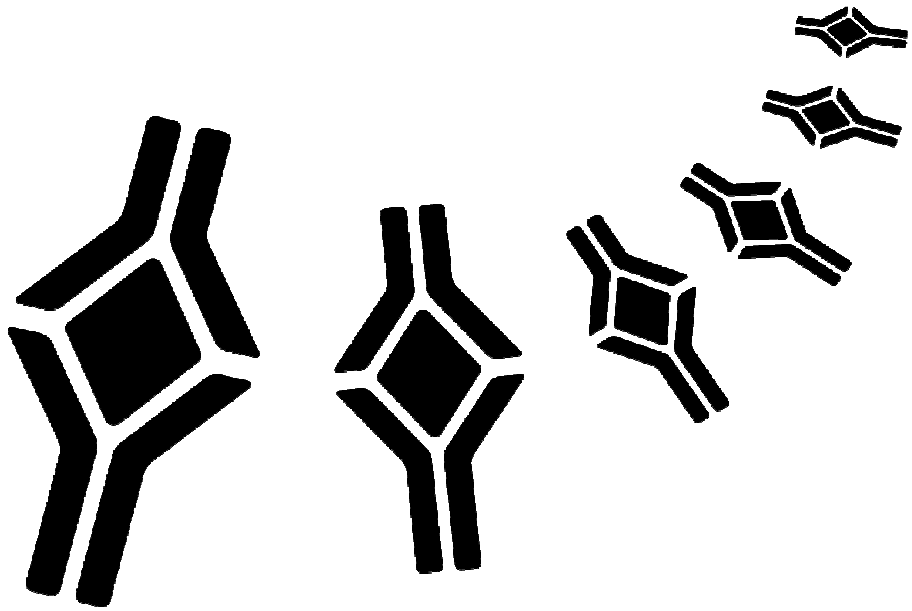
Research
and Diagnostic Products



# HUMAN PANCREATIC-DERIVED FACTOR ELISA

**Product Data Sheet**

**Cat. No.: RD191259200R**

**For Research Use Only**

# CONTENTS



» **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 RD191259200R Human Pancreatic-Derived Factor ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human pancreatic-derived factor protein (PANDER).

### Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures human PANDER in serum, citrate, heparin and EDTA plasma
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

# 2. STORAGE, EXPIRATION

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

Pancreatic-derived factor (FAM3B, PANDER) consists of 235 amino acids and is a member of the family FAM3 proteins. Pander has a characteristic secondary structure: quadruple helix topology up-up-down-down, which is stabilized by two disulfide bonds between cysteine [1].
The human PANDER is highly expressed in the pancreas. In mice PANDER is highly expressed in the pancreas and to a much lesser extent in the small intestine and prostate, its occurrence in the muscles and liver has not been clearly demonstrated.
Immunohistochemically PANDER was selectively detected in Purkinje cells of the Cerebellum, brain and nerve cell bodies of the brain stem [2]. Expression of the PANDER in the pancreas has been shown specifically in the pancreatic islets of α and β cells. PANDER is included along with insulin in secretory vesicles of β cells [3, 4]. The occurrence in α cells is linked to glucagon secretion [5]. PANDER is directly connected with the regulation of glucose produced by the liver and with glucose used in human metabolism. In connection with the concentration of glucose, increased production of PANDER by β cells depends on the levels of proinflammatory cytokines interleukin 1β, tumor necrosis factor α and interferon γ. Unlike β cells, changes in the levels of glucose in α cells do not cause any effect on the production of PANDER, but there was an increase in PANDER recorded in conjunction with low levels of insulin [1].
A negative effect of PANDER is its participation in the formation and subsequent dysfunction of apoptosis to necrosis of pancreatic β cells [6].
Experiments on mice have shown that PANDER regulates glucose output by the liver. During fasting, production of PANDER supports hyperglycemia, compensatory hyperinsulinemia, decreased triglycerides and increased levels of corticosterone [2].
Because PANDER is associated with insulin production and action, we can expect higher levels of PANDER in patients with metabolic syndrome, glucose intolerance and type 2 diabetes.

<u>Areas of investigation:</u>
Energy metabolism and body weight regulation
Metabolic syndrome
Type 2 diabetes

## 4. TEST PRINCIPLE

In the BioVendor Human Pancreatic-Derived Factor ELISA, standards and samples are incubated in microtiter plate wells pre-coated with polyclonal anti-human PANDER antibody. After 60 minutes incubation followed by washing, biotin labelled polyclonal anti-human PANDER antibody is added to the wells and incubated for 60 minutes at room temperature with the captured human PANDER. Following another washing step, the remaining streptavidin-HRP conjugate is added. After 60 minutes incubation at room temperature 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 PANDER. A standard curve is constructed by plotting absorbance values against PANDER 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 immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- The kit is intended to determine PANDER in biological material. Such material 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 | lyophilized | 2 vials |
| Streptavidin-HRP Conjugate | ready to use | 13 ml |
| Master Standard | lyophilized | 2 vials |
| Biotin-Ab Diluent | ready to use | 13 ml |
| Dilution Buffer | ready to use | 2 x 20 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

- 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
- Plate cover
- 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**

- **Assay reagents supplied ready to use:**

**Antibody Coated Microtiter Strips**
Stability and storage:
Return the unused strips to the provided aluminum 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**
**Biotin-Ab Diluent**
**Streptavidin-HRP Conjugate**
**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 PANDER Master Standard:**
**Refer to the 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 PANDER in the stock solution is **4 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

| Volume of Standard | Dilution Buffer | Concentration |
|---|---|---|
| Stock | - | 4 ng/ml |
| 500 µl of stock | 500 µl | 2 ng/ml |
| 500 µl of 2 ng/ml | 500 µl | 1 ng/ml |
| 500 µl of 1 ng/ml | 500 µl | 0.5 ng/ml |
| 500 µl of 0.5 ng/ml | 500 µl | 0.25 ng/ml |
| 500 µl of 0.25 ng/ml | 500 µl | 0.125 ng/ml |

**Prepared Standards are ready to use, do not dilute them.**
Stability and storage:
**Do not store the diluted Standard solutions.**

**Biotin Labelled Antibody**
**Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!**
Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Dilute Biotin Labelled Antibody Concentrate 100x with Biotin-Ab Diluent (e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Biotin-Ab Diluent for 8 wells).
Stability and storage:
**Do not store diluted Biotin Labelled Antibody working 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

The kit measures human PANDER in serum, citrate, heparin and EDTA plasma.

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.

Dilute samples 10x with Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 135 µl of Dilution Buffer for singlets, or preferably 30 µl of sample + 270 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

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

*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, 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 with cover at room temperature (ca. 25°C) for **60 minutes**, 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 with cover at room temperature (ca. 25°C) for **60 minutes**, 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 with cover at room temperature (ca. 25°C) for **60 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 (ca. 25°C). The incubation time may be extended [up to 20 minutes] if the reaction temperature is less than 20°C. Do not shake the plate during this 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 PANDER 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 4.0** | Sample 1 | Sample 9 | Sample 17 | Sample 25 | Sample 33 |
| **B** | **Standard 2.0** | Sample 2 | Sample 10 | Sample 18 | Sample 26 | Sample 34 |
| **C** | **Standard 1.0** | Sample 3 | Sample 11 | Sample 19 | Sample 27 | Sample 35 |
| **D** | **Standard 0.5** | Sample 4 | Sample 12 | Sample 20 | Sample 28 | Sample 36 |
| **E** | **Standard 0.25** | Sample 5 | Sample 13 | Sample 21 | Sample 29 | Sample 37 |
| **F** | **Standard 0.125** | Sample 6 | Sample 14 | Sample 22 | Sample 30 | Sample 38 |
| **G** | **Blank** | Sample 7 | Sample 15 | Sample 23 | Sample 31 | Sample 39 |
| **H** | **Blank** | Sample 8 | Sample 16 | Sample 24 | Sample 32 | Sample 40 |

*Figure 1: Example of a work sheet.*

## 12. CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the absorbance (Y) of Standards against the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of PANDER (ng/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. 1.5 ng/ml (from standard curve) x 10 (dilution factor) = 15 ng/ml.**

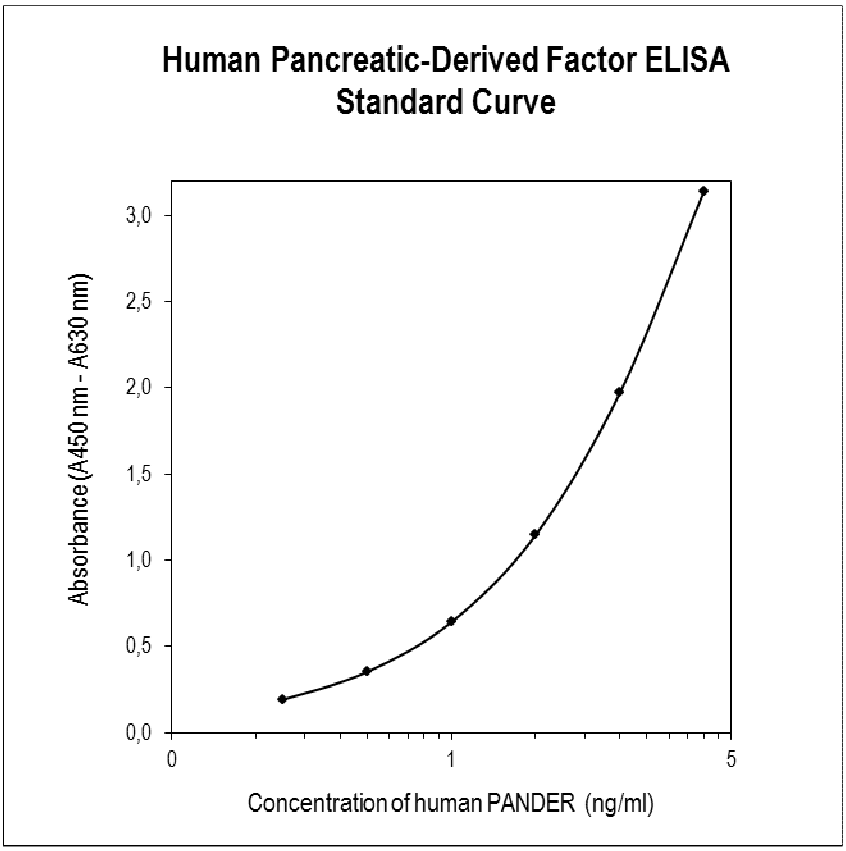



*Figure 2: Typical Standard Curve for Human Pancreatic-Derived Factor ELISA.*

# 13. PERFORMANCE CHARACTERISTICS

**Typical analytical data of BioVendor Human Pancreatic-Derived Factor 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: $A_{blank}$ + $3xSD_{blank}$) is calculated from the real PANDER values in wells and is: 0.037 ng/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 are specific for human PANDER with no detectable crossreactivities to the following proteins: FAM3A, FAM3C, FAM3D.
We observed no interference of hemoglobin (1.0 mg/ml), bilirubin (170 µmol/l) and triglycerides (5.0 mmol/l) on the measurement of human PANDER.

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

| *Mammalian serum Sample* | *Observed Crossreactivity* |
|---|---|
| Bovine | no |
| Cat | no |
| Dog | yes |
| Goat | no |
| Hamster | no |
| Horse | yes |
| Monkey | yes |
| Mouse | no |
| Rabbit | no |
| Rat | no |
| Sheep | no |
| Pig | yes |

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

| *Sample* | *Mean (ng/ml)* | *SD (ng/ml)* | *CV (%)* |
|---|---|---|---|
| 1 | 5.92 | 0.37 | 6.29 |
| 2 | 12.36 | 0.52 | 4.18 |

Inter-assay (Run-to-Run) (n=5)

| *Sample* | *Mean (ng/ml)* | *SD (ng/ml)* | *CV (%)* |
|---|---|---|---|
| 1 | 6.23 | 0.39 | 6.31 |
| 2 | 10.40 | 0.44 | 4.28 |

- **Spiking Recovery**

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

| *Sample* | ***O**bserved (ng/ml)* | ***E**xpected (ng/ml)* | *Recovery **O/E** (%)* |
|---|---|---|---|
| 1 | 7.59 | - | - |
| | 11.94 | 12.59 | 94.8 |
| | 17.33 | 17.59 | 98.5 |
| | 22.72 | 22.59 | 100.6 |
| 2 | 12.23 | - | - |
| | 17.73 | 17.23 | 102.9 |
| | 22.55 | 22.23 | 101.4 |
| | 26.87 | 27.23 | 98.7 |

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

| *Sample* | *Dilution* | ***O**bserved (ng/ml)* | ***E**xpected (ng/ml)* | *Recovery **O/E** (%)* |
|---|---|---|---|---|
| 1 | - | 7.99 | - | - |
| | 2x | 3.67 | 3.99 | 91.9 |
| | 4x | 1.92 | 2.00 | 96.2 |
| | 8x | 0.95 | 1.00 | 95.2 |
| 2 | - | 12.97 | - | - |
| | 2x | 6.41 | 6.49 | 98.8 |
| | 4x | 3.27 | 3.24 | 100.8 |
| | 8x | 1.56 | 1.62 | 96.2 |

- **Effect of sample matrix**

Heparin, citrate and EDTA plasmas were compared to respective serum samples from the same 10 individuals.
Results are shown below:

| *Volunteer No.* | *Serum (ng/ml)* | *Plasma (ng/ml)* | | |
|---|---|---|---|---|
| | | *EDTA* | *Citrate* | *Heparin* |
| 1 | 29.67 | 28.51 | 28.90 | 31.32 |
| 2 | 29.14 | 25.39 | 25.42 | 30.80 |
| 3 | 26.05 | 27.40 | 26.10 | 29.70 |
| 4 | 2.07 | 1.85 | 1.73 | 2.03 |
| 5 | 0.70 | 0.56 | 0.50 | 0.74 |
| 6 | 0.28 | 0.31 | 0.13 | 0.17 |
| 7 | 3.53 | 3.36 | 3.25 | 3.21 |
| 8 | 2.48 | 1.72 | 2.62 | 1.95 |
| 9 | 0.42 | 0.60 | 0.52 | 0.35 |
| 10 | 3.77 | 3.43 | 4.31 | 3.48 |
| **Mean (ng/ml)** | **9.81** | **9.31** | **9.35** | **10.37** |
| **Mean Plasma/Serum (%)** | | **95** | **95** | **106** |
| **Coefficient of determination $R^2$** | | **0.99** | **0.99** | **0.99** |

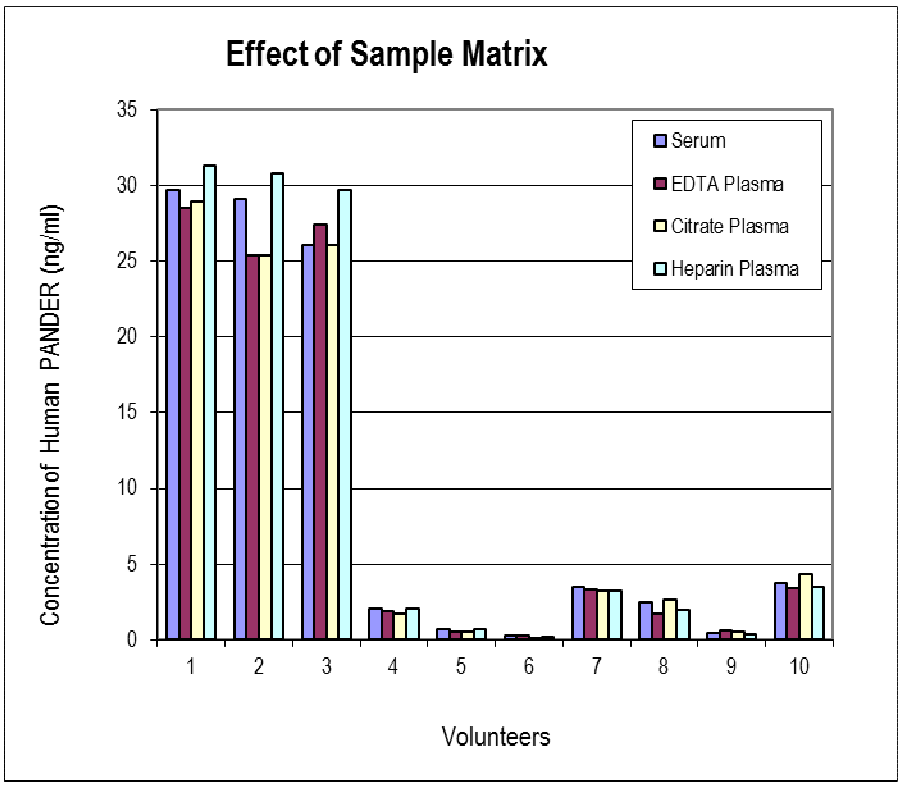



*Figure 3: PANDER levels measured using Human Pancreatic-Derived Factor ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.*

## 14. DEFINITION OF THE STANDARD

In this assay as the standard recombinant Human Pancreatic-Derived Factor is used. Recombinant human Pancreatic-Derived Factor is consisting of 206 amino-acid residues of Pancreatic-Derived Factor and 10 additional amino-acids. The apparent molecular weight is 24 kDa .

## 15. PRELIMINARY DATA

The following results were obtained when serum samples from 148 blood donors (83 men + 65 women, 20-65 years old) were assayed with the BioVendor Human Pancreatic-Derived Factor ELISA in our laboratory:
Normal range comprised PANDER mean concentration 12.2 ng/ml (SD = 34.1).

The data quoted in these instructions should be used for guidance only. Each laboratory should establish its own normal and pathological ranges for PANDER levels with the assay. Each laboratory should establish a quality control program to monitor the quality of the assay.

## 16. METHOD COMPARISON

BioVendor Human Pancreatic-Derived Factor ELISA has not been compared to any other immunoassay.

# 17. 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 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 or samples

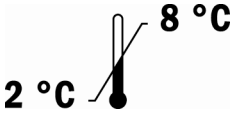
## 18. REFERENCES

### References to human PANDER :


1. Wilson, G.W., Robert-Cooperman, C.E., Burkhardt, B.R.: "PANcreatic-DERived factor: Novel hormone PANDERing to glucose regulation." FEBS Letters. 2011, 585:2137-2143.
2. Wilson, G.W., Schupp, M., Burkhardt, B.R., Wu, J., Young, R.A., Wolf, B.A.: "Livers-Specific Overexpression of Pancreatic-Derived Factor (PANDER) Induces Fasting Hyperglycemia in Mice." Endocrinology 2010, 151(11):5174-5184.
3. Robert-Cooperman, C.E., Carnegie, J.R., Wilson, G.W., Yang, J., Cook, J.R., Wu, J., Young, R.A., Wolf, B.A., Burghardt, B.R.: "Targeted Disruption of Pancreatic-Derived Factor (PANDER, FAM3B) Impairs Pancreatic β-Cell Function." Diabetes 2010, 59:2209-2218.
4. Yang, J., Robert, C.E., Burkhardt, B.R., Young, R.A., Wu, J., Gao, Z., Wolf, B.A.: "Mechanism of Glucose-Induced Secretion of Pancreatic-Derived Factor (PANDER or FAM3B) in Pancreatic β-Cells." Diabetes 2005, 54:3217-3228.
5. Carnegie, J.R., Robert-Cooperman, C.E., Wu, J., Young, R.A., Wolf, B.A., Burkhardt, B.R.: "Characterization of the Expression, Localization and Secretion of PANDER in α-Cells." Moll. Cell. Endocrinol. 2010, 325(1-2):36-45.
6. Wang, C., Guan, Y., Yang, J.: "Cytokines in the Progression of Pancreatic β-Cell Dysfunction." Int. J. Endocrinol. 2010, ID 515136:1-10.


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

## 19. EXPLANATION OF SYMBOLS

| | |
|---|---|
| REF | Catalogue number |
| Cont. | Content |
| LOT | Lot number |
|  | See instructions for use |
|  | Expiry date |
|   | Storage conditions |
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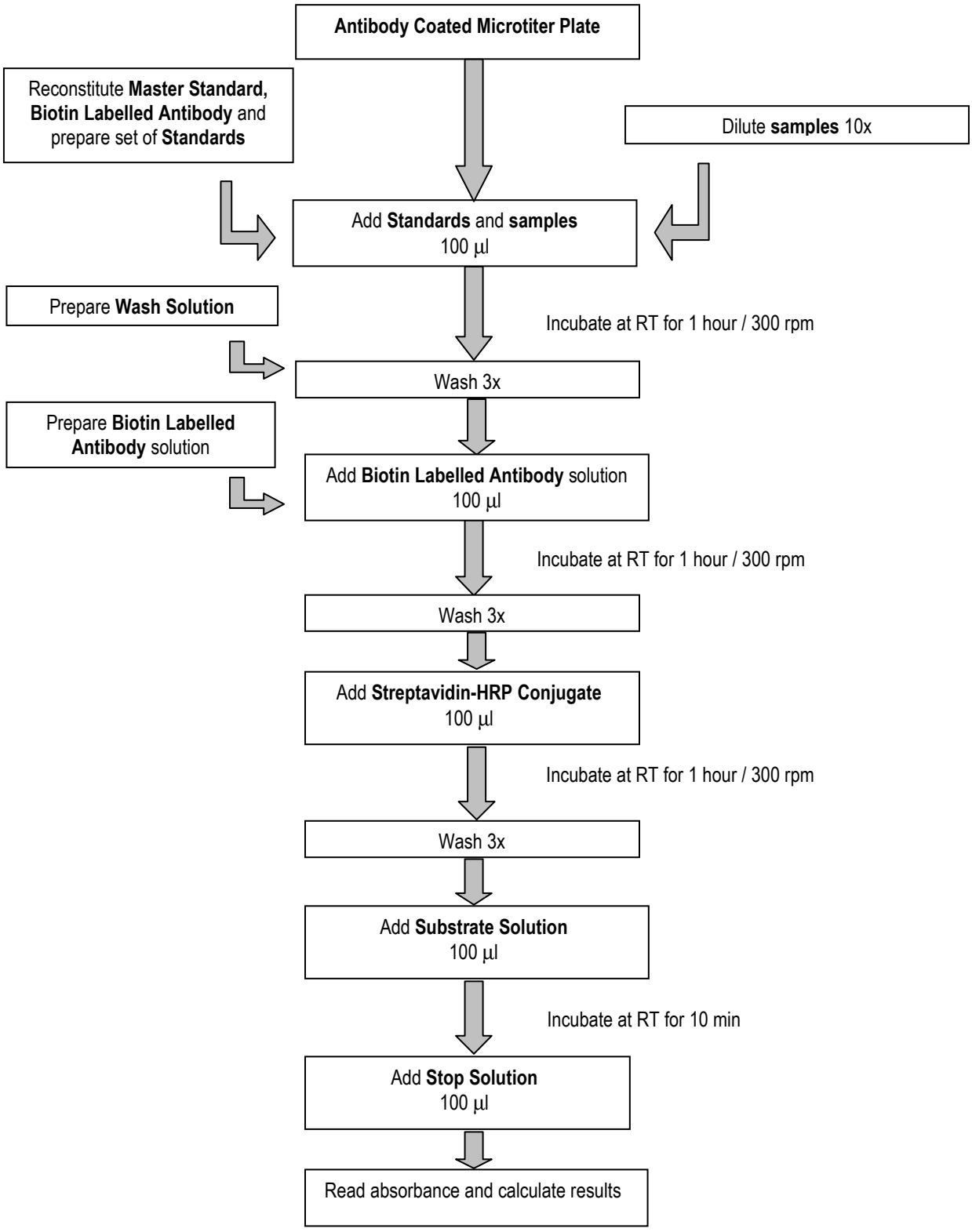
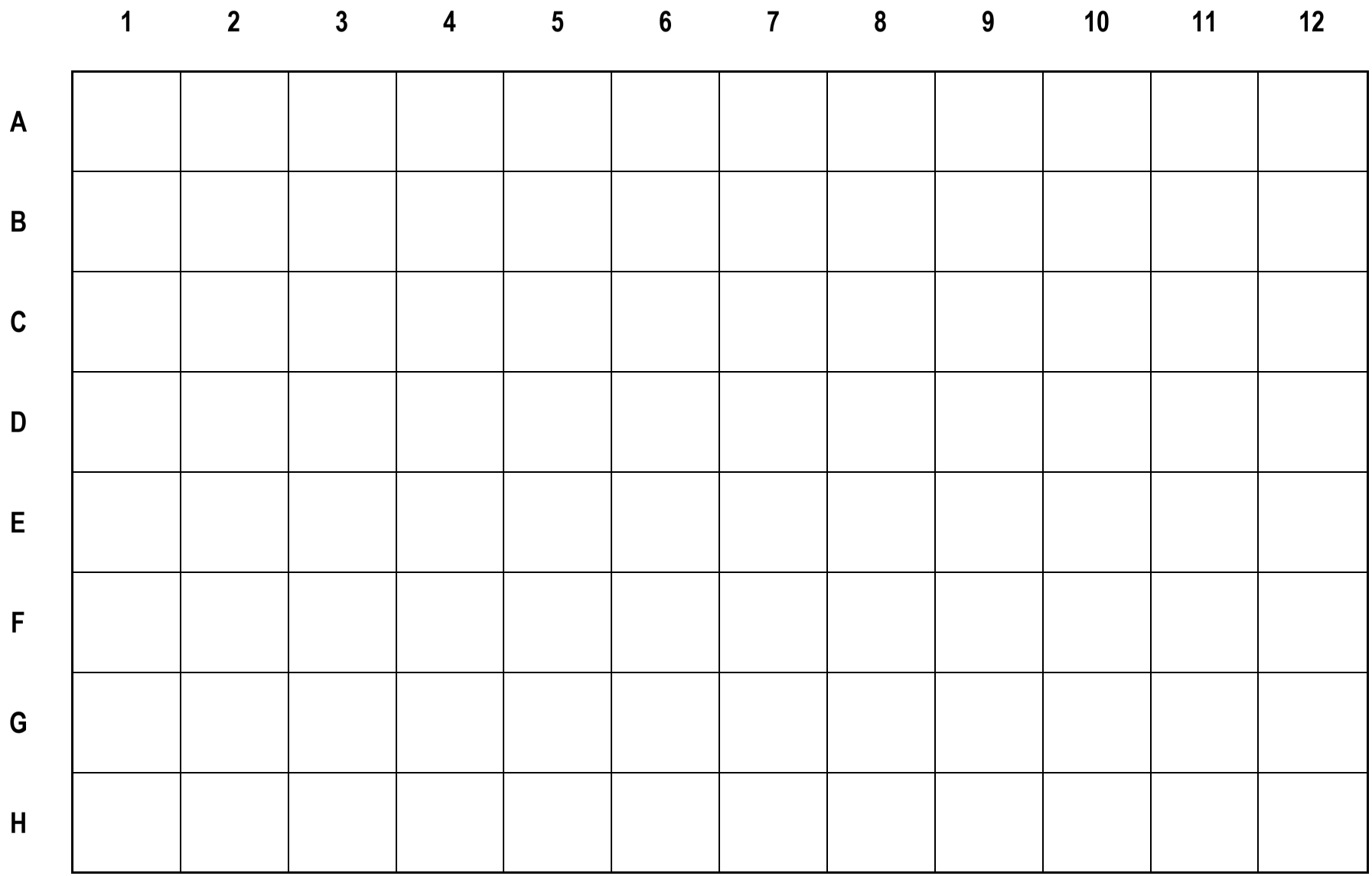
## Assay Procedure Summary

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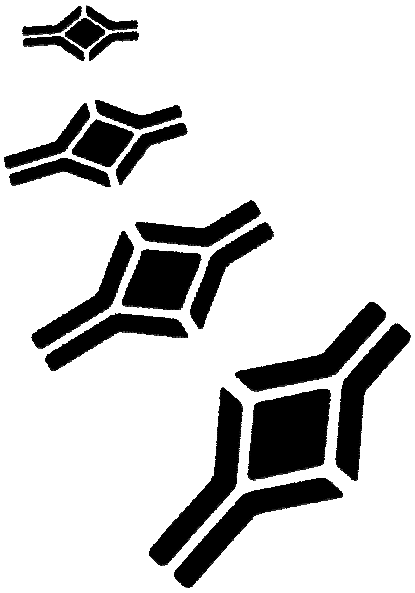


| | | | | |
|---|---|---|---|---|
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