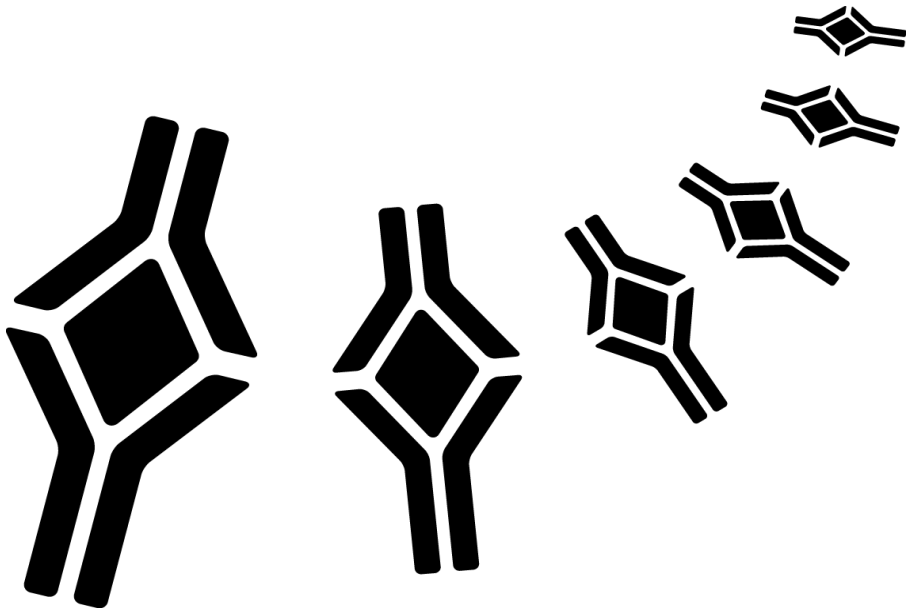
BioVendor
Research
and Diagnostic Products



HUMAN NESFATIN-1 ELISA

Product Data Sheet

Cat. No.: RD191227200R

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 RD191227200R Human Nesfatin-1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human nesfatin-1.

›› Features

- **It is intended for research use only**
- The total assay time is less than 4 hours
- The kit measures human nesfatin-1 in serum, plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is recombinant (E.coli) 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

Nesfatin-1 was first described in 2006 as a neurohormone derived from the protein NEFA/nucleobindin2 (NUCB2). It is a polypeptide consisting of 82 amino acids, with a high level of homology between human, mouse and rat. Nesfatin-1 has been localized in the brain--particularly in the nuclei of the hypothalamus, adipose tissue and the gastrointestinal tract.It is also found in serum and cerebrospinal fluid. Nesfatin-1 was identified as a satiety molecule affecting fat metabolism. Intracerebroventricular administration of nesfatin-1 induces a decrease in food intake and body weight and an increase in sympathetic nerve activity and mean arterial pressure. The effect of Nesfatin-1 is leptin – independent and is likely mediated by the melanocortin system. Several studies are currently focused on the possibility of using Nesfatin-1 in the treatment of obesity, even in individuals with leptin resistance. Serum Nesfatin-1 could also serve as a clinical marker in the diagnosis of many diseases.
The important role of nesfatin-1 in the metabolism of glucose and insulin has been reported in several studies. Different concentrations were observed in patients with diabetes mellitus type I and II. In individuals with DM type II postprandial concentration of nesfatin-1 was significantly

lower than levels in individuals with DM type I and healthy subjects. Different levels of Nesfatin-1 were measured in patients with anorexia nervosa and panic disorders in comparison to a healthy control group.
Data obtained in experiments with rat models indicate that nesfatin-1 acts as a peripheral modulator of the cardiac function.
Much higher levels of serum Nesfatin-1 (up to 160x) were detected in patients with newly-diagnosed epilepsy. During the treatment with anti-epileptics concentration of Nesfatin-1 decreased, but it was still about 10 times higher than the level of the hormone in healthy individuals. This suggests that it might be possible to use the Nesfatin-1 as a marker for diagnosis and monitoring of epilepsy.

Areas of investigation:
Energy metabolism and body weight regulation
Metabolic syndrome
Diabetology
Endocrinology
Neuroscience

4. TEST PRINCIPLE

In the BioVendor Human Nesfatin-1 ELISA, standards and samples are incubated in microplate wells pre-coated with sheep polyclonal anti-human Nesfatin-1 antibody. After 1 hour incubation at room temperature and washing, biotin labelled polyclonal anti-human Nesfatin-1 antibody is added to the wells and incubated for 60 minutes with captured Nesfatin-1. 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 Nesfatin-1. 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

- **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 may contain components of human or animal origin. These materials should be handled as potentially infectious
- 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. REAGENTS 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
Dilution Buffer	ready to use	50 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
- 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 aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

Dilution Buffer
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 Nesfatin-1 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 Nesfatin-1 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
250 µl of stock	250 µl	2 ng/ml
250 µl of 2 ng/ml	250 µl	1 ng/ml
250 µl of 1 ng/ml	250 µl	0.5 ng/ml
250 µl of 0.5 ng/ml	250 µl	0.25 ng/ml
250 µl of 0.25 ng/ml	250 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 Dilution Buffer needed for reconstitution of Biotin Labeled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Prepare the working Biotin Labelled Antibody solution by adding 1 part of Biotin Labelled Antibody Concentrate (100x) to 99 parts of Dilution Buffer.
Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Dilution Buffer, for 1 strip (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 Nesfatin-1 in serum, plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thawed samples thoroughly just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute serum or plasma samples 5x with Dilution Buffer just prior to the assay (e.g. 30 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 50 µl of sample + 200 µl of Dilution Buffer for duplicates). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°, 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 diluted Standards, 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 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-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 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-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 min**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 5-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 1: 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 Nesfatin-1 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**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 2**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 1**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 0.5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 0.25**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 0.125**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
H	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microplate 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 Nesfatin-1 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. 2 ng/ml (from standard curve) x 5 (dilution factor) = 10 ng/ml

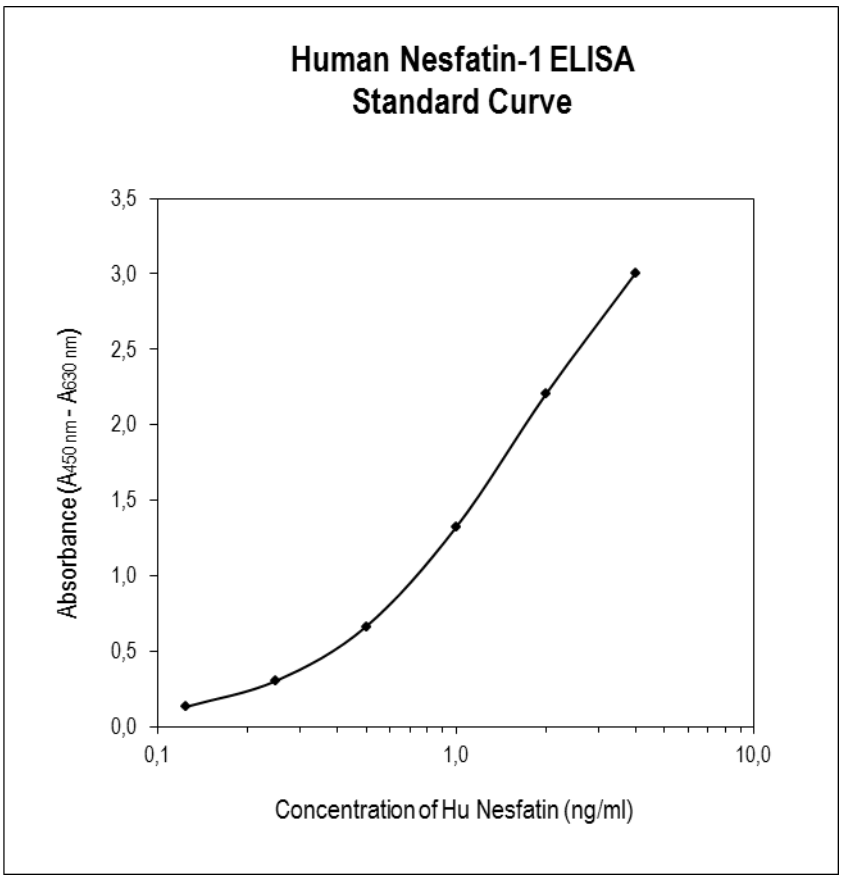


Figure 2: Typical Standard curve for Human Nesfatin-1 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Nesfatin-1 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 Nesfatin-1 values in wells and is 0.021 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 sample calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**

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	no
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	yes
Rabbit	no
Rat	no
Sheep	no

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

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	6.07	0.17	2.75
2	0.83	0.05	5.75

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	3.81	0.21	5.43
2	6.89	0.43	6.29

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Nesfatin-1 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	8.82	-	-
	11.45	11.32	101.1
	14.73	13.82	106.6
	20.84	18.82	110.8
2	4.54	-	-
	6.42	7.04	91.1
	8.86	9.54	92.9
	14.12	14.54	97.1

- **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	-	13.10	-	-
	2x	6.45	6.55	98.5
	4x	3.45	3.28	105.3
	8x	1.80	1.64	109.9
2	-	8.35	-	-
	2x	4.35	4.18	104.2
	4x	2.40	2.09	115.0
	8x	1.20	1.04	115.0

- **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	3.48	3.58	3.37	3.88
2	0.25	0.29	0.12	0.27
3	3.47	3.44	2.47	3.52
4	0.81	1.12	0.87	0.99
5	0.52	0.50	0.45	0.45
6	1.14	1.09	0.97	1.23
7	2.65	2.87	2.39	1.53
8	1.05	0.95	0.82	1.03
9	1.09	0.95	0.79	1.00
10	1.29	1.86	1.05	1.69
Mean (ng/ml)	**1.58**	**1.66**	**1.33**	**1.56**
Mean Plasma/Serum (%)		**105.5**	**84.3**	**98.7**
Coefficient of determination R^2		**0.97**	**0.95**	**0.88**

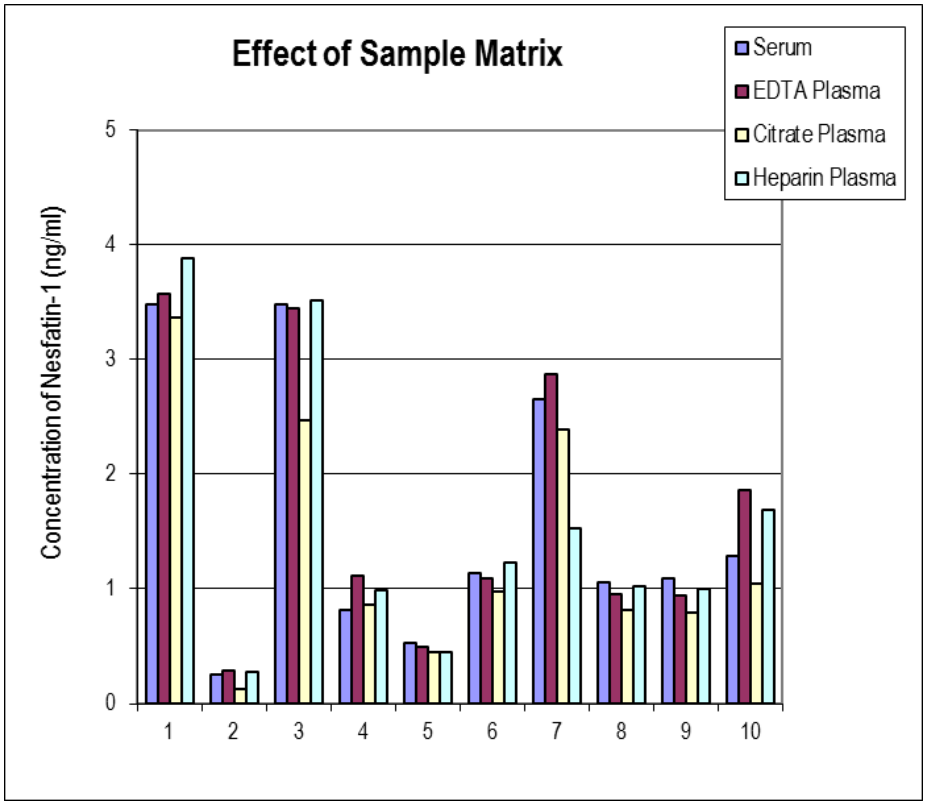


Figure 3: Nesfatin-1 levels measured using Human Nesfatin-1 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is a recombinant human Nesfatin-1. Recombinant Human Nesfatin-1 (AA 1-92, produced in E. coli) is a 10.79 kDa protein containing 82 amino acids of human Nesfatin-1 and 10 amino acids in addition.

15. PRELIMINARY POPULATION DATA

The reference range of serum samples from healthy volunteers (N = 168) has been determined using this Human Nesfatin-1 ELISA kit in our laboratory:
Mean concentration of Nesfatin-1 from this healthy population was 1.77 ng/ml (SD = 3.8).

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for human Nesfatin-1 levels with the assay.

16. 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

17. REFERENCES

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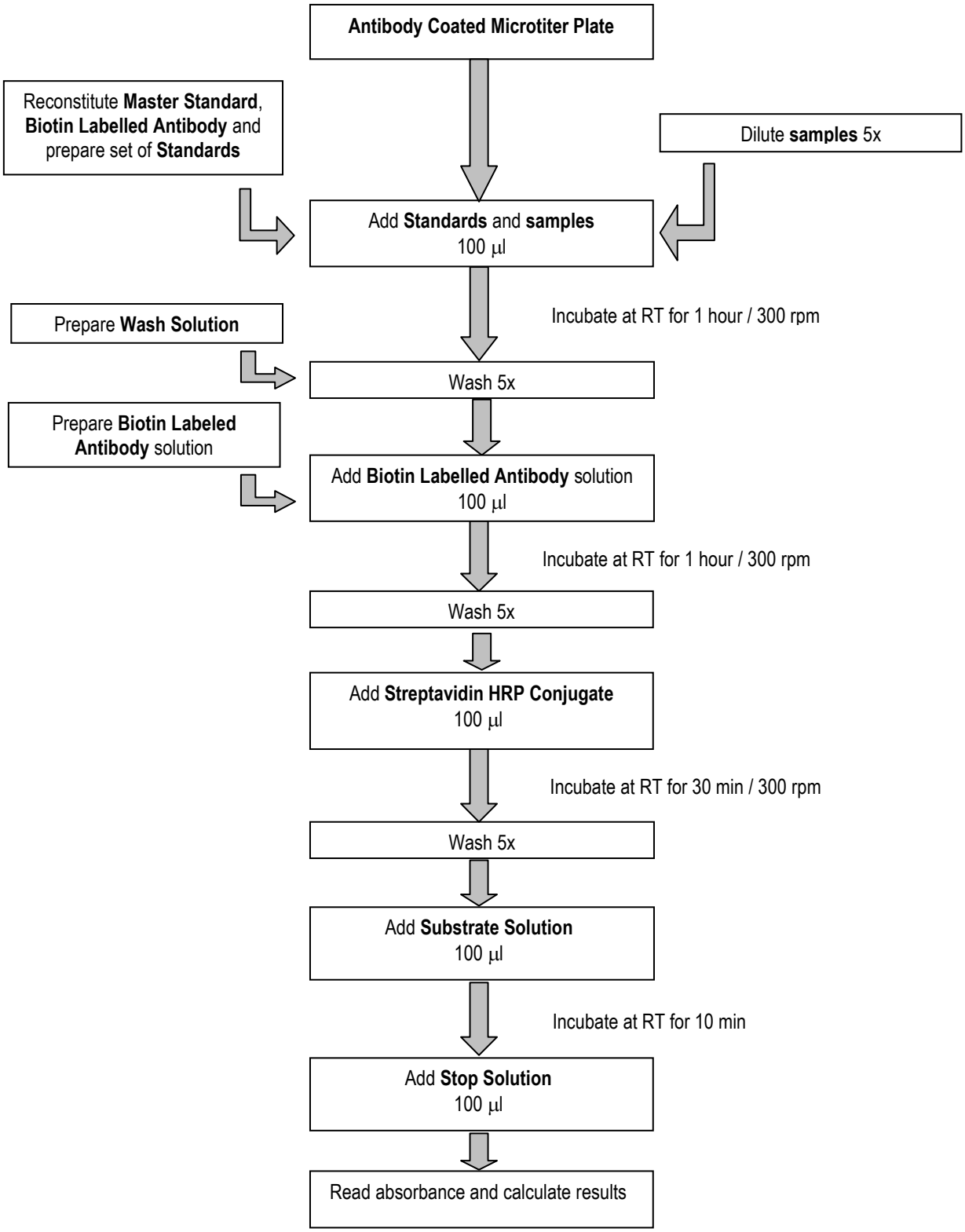
- Riva M. et a.: Nesfatin-1 stimulates glucagon and insulin secretion and beta cell NUCB2 is reduced in human type 2 diabetic subjects. Cell Tissue Res., 2011.

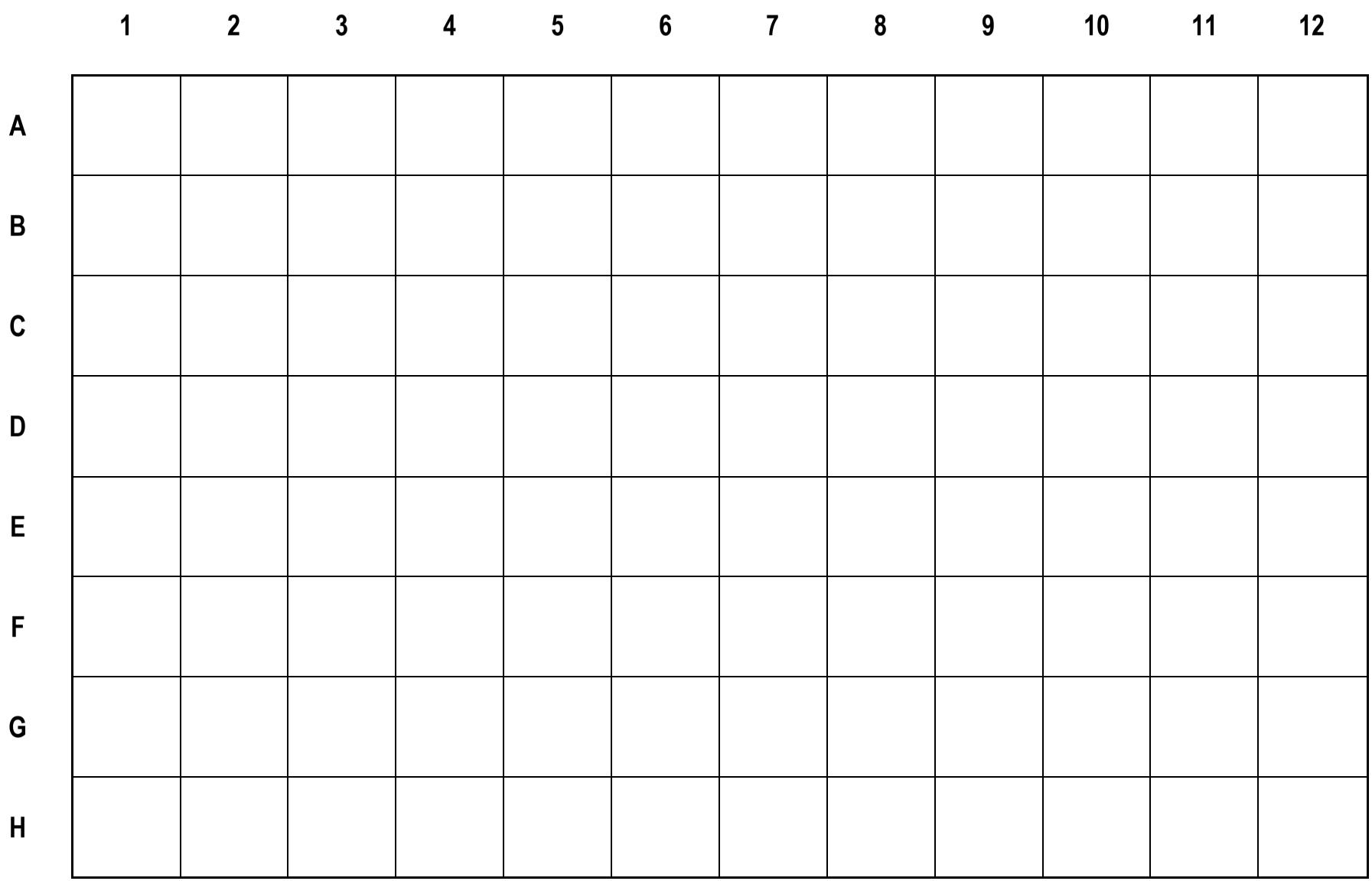
» **For more references on this product see our WebPages at www.biovendor.com**

18. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary

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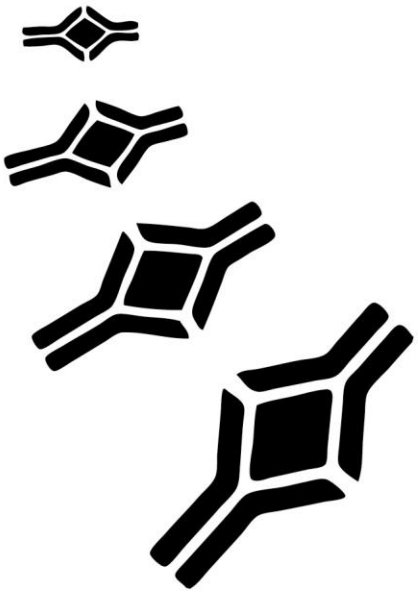

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