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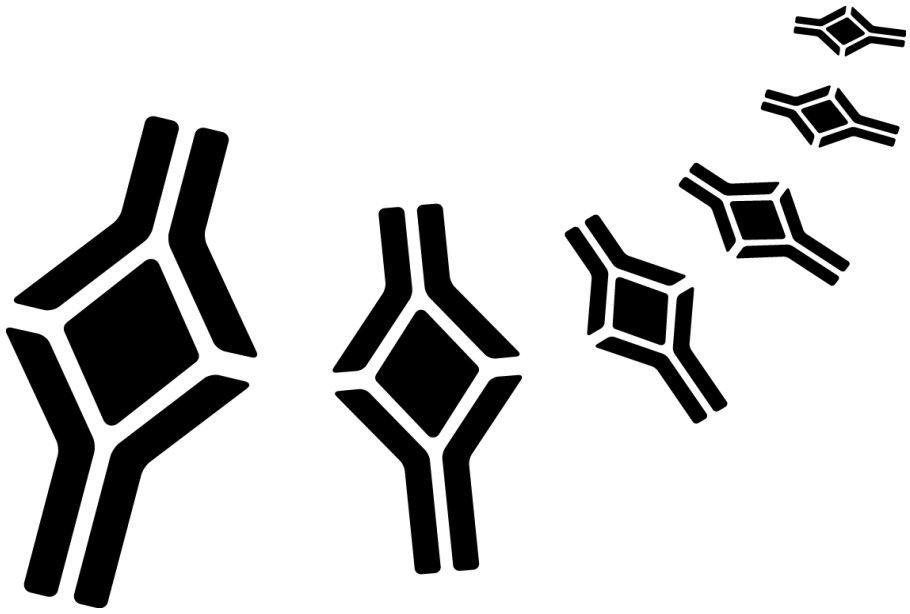
Research
and Diagnostic Products



HUMAN GASTROKINE 1 ELISA

Product Data Sheet

Cat. No.: RD191268200R

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 RD191268200R Human Gastrokine 1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human gastrokine 1 (GKN1).

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures GKN1 in serum and plasma (EDTA, citrate, heparin)
- 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

Gastrokine 1, previously known as AMP-18, CA11, FOVEOLIN and TFIZ, was formally named "GKN1" by the HUGO gene Nomenclature Committee for its gastric-specific expression and its highly conserved presence in the gastric mucosa of many mammalian species. [2] Gastrokine 1 is a novel protein that was firstly cloned by a Japanese group in 2000. [7]
GKN1 belongs to a family of genes encoding stomach-specific secreted proteins consisting of 3 known members: gastrokine 1 (GKN1), gastrokine 2 (GKN2) and gastrokine 3 (GKN3). [8]
GKN1 gene of about 6 kb was reported to be located at 2p13 and contains 6 exons. The GKN1 gene encodes a small protein of 185 amino acids containing a N-terminal signal peptide. [8] It is a secreted protein with a molecular weight of approximately 18 kDa. [7] GKN1 protein contains a BRICHOS domain, which is associated with dementia, respiratory distress and cancer. [6] Molecular studies on the BRICHOS domain function have suggested that it has a range of possible roles, including intracellular trafficking, propeptide processing, chaperone function and secretion. However, the biological activities of the BRICHOS domain in GKN1 have not been elucidated. [2]
Gastrokine 1 has been detected recently in normal gastric mucosa, but not in other regions of the gastrointestinal tract. [1] Immunoelectron microscopy indicated that the GKN1 protein is localized within the granules just under the apical plasma membrane. [2] These expression patterns of GKN1 indicate that it may play a distinctive role in the stomach. [7] Functionally, GKN1 promotes the maturation of gastric mucosa, and maintains the integrity of gastric mucosal epithelium through mitogenic and mutagenic abilities. GKN1 may also protect the intestinal mucosal barrier by acting on specific tight junction proteins and stabilizing perijunctional actin. [6]
If the protein is downregulated, the repair process may be impaired. Recently, a number of studies have found that deficiency of GKN1 can result in instability of the gastric epithelium. [1] Clinically, GKN1 is downregulated in Helicobacter pylori-infected gastric epithelial cells, and this loss of GKN1 expression is detected in gastric cancer and precancerous lesion, such as intestinal metaplasia. [4] Invasive factor such as H. pylori contribute to the downregulation of GKN1, whilst inducing ulceration and cancer. [1] Moreover, GKN1 plays an important role in epithelial-mesenchymal transition and migration of gastric cancer cells by regulating reactive oxygen species (ROS) and the P13K/Akt pathway. Recently, it has been suggested that GKN1 induces senescence through activating p16/Rb pathway in gastric cancer cells. [4] Thus, GKN1 may play a key role in the progression of gastric cancer, and may be a potential biomarker for the early detection of gastric cancer. [1]

<u>Areas of investigation:</u>
Oncology
Immune Response, Infection and Inflammation

4. TEST PRINCIPLE

In the BioVendor Human Gastrokine 1 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human GKN1 antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human GKN1 antibody is added and incubated for 60 minutes with captured human GKN1. 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 GKN1. 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. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	2x20ml
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 5-1000 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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate
Dilution Buffer
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 GKN1 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 GKN1 in the stock solution is **32 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	32 ng/ml
250 µl of stock	250 µl	16 ng/ml
250 µl of 16 ng/ml	250 µl	8 ng/ml
250 µl of 8 ng/ml	250 µl	4 ng/ml
250 µl of 4 ng/ml	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

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.

Biotin Labelled Antibody Conc. (100x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Conc. (100x) with 99 parts Dilution Buffer.
Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Conjugate Diluent for 1 strip (8 wells). **Mix well** (not to foam).
Stability and storage:
Opened Biotin Labelled Antibody Concentrate (100x) is stable 3 months when stored at 2–8°C.
Do not store the diluted Biotin Labelled Antibody Solution.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. e.g. 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 GKN1 in serum and plasma (EDTA, citrate, heparin).

Samples can be assayed immediately after collection, or after long-term storage at - 20°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 **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.
Stability and storage:
Samples should be stored at -20°, or preferably at -70°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 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. Pipet **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 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. Pipet **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 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. 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: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine GKN1 concentration of off-scale samples. The readings at 405 nm should not replace the reading 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 32**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 16**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 8**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 4**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 2**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 1**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 0.5**	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 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 GKN1 (ng/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of 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. 4 ng/ml (from standard curve) x 3 (dilution factor) = 12 ng/ml.

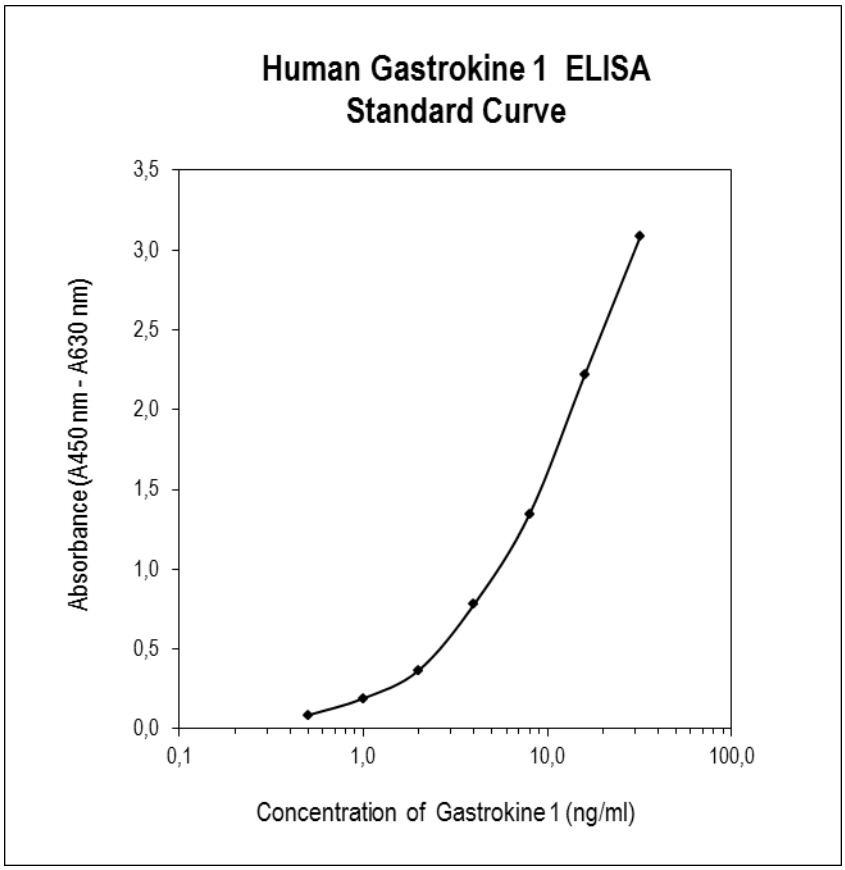


Figure 2: Typical Standard Curve for Human Gastrokine 1 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Gastrokine 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 GKN1 values in wells and is 0.035 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.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	3.10	0.07	2.3
2	12.29	0.27	2.2

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	7.01	0.60	8.0
2	21.85	1.70	7.6

- **Spiking Recovery**

Serum samples were spiked with different amounts of human GKN1 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	2.68	-	-
	4.65	4.55	102.2
	6.58	6.43	102.3
	10.43	10.18	102.5
2	3.31	-	-
	5.21	5.19	100.3
	7.08	7.06	100.3
	11.15	10.81	103.1

- **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	-	7.56	-	-
	2x	4.10	3.78	108.4
	4x	2.03	1.89	107.3
	8x	1.10	0.95	115.8
2	-	15.20	-	-
	2x	8.38	7.60	110.2
	4x	4.11	3.80	108.1
	8x	1.88	1.90	98.7

- **Effect of sample matrix**

Citrate, heparin 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	3.95	4.25	3.39	3.88
2	8.03	8.05	7.14	7.94
3	3.95	3.79	3.50	3.86
4	9.42	10.42	10.06	11.41
5	2.27	3.05	1.99	2.31
6	3.35	3.18	2.45	2.88
7	5.60	5.52	4.62	5.52
8	4.36	4.51	3.82	4.21
9	3.19	3.67	3.22	3.49
10	3.80	3.65	2.84	3.44
Mean (ng/ml)	**4.79**	**5.01**	**4.30**	**4.89**
Mean Plasma/Serum (%)	**-**	**104.5%**	**89,8%**	**102.2%**
Coefficient of determination R^2	**-**	**0.97**	**0.96**	**0.97**

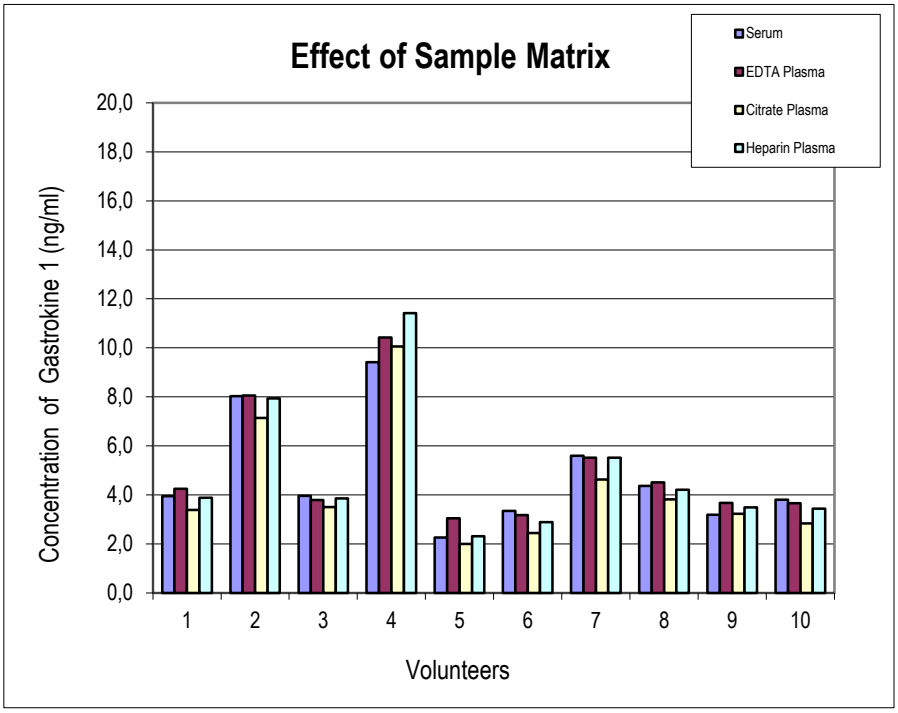


Figure 3: GKN1 levels measured using Human Gastrokine 1 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is recombinant protein. The recombinant human GKN1, produced in *E. coli*, is protein consisting of 175 amino-acid residues.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 154 unselected donors (87 men + 67 women) 21 – 65 years old were assayed with the Biovendor Human Gastrokine 1 ELISA in our laboratory.

Sex	*Age (years)*	*n*	*GKN1(ng(ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	21-29	17	5.47	5.35	1.28	3.33	8.23
	30-39	27	6.37	6.58	1.53	3.92	11.82
	40-49	30	6.76	6.61	1.56	3.71	10.52
	50-65	13	7.08	7.03	1.87	3.17	10.71
Women	22-29	11	6.48	6.56	1.06	4.63	8.20
	30-39	26	6.62	6.34	1.39	3.71	10.12
	40-49	22	6.08	6.03	1.38	3.92	9.15
	50-61	8	7.91	8.12	2.53	3.55	11.26

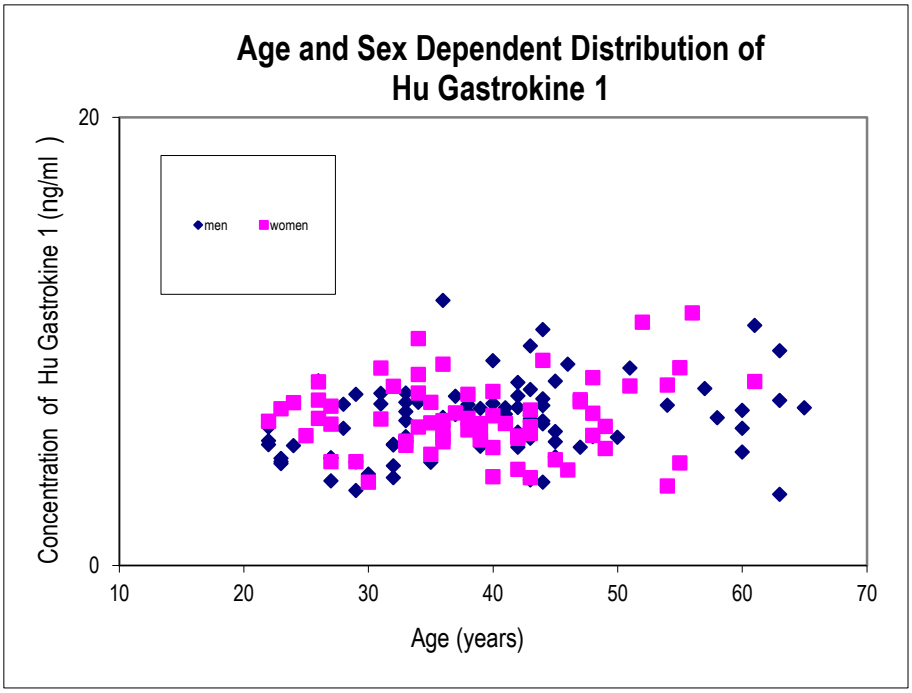


Figure 4: Human GKN1 concentration plotted against donor age and sex.

- **Reference range**

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 GKN1 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Gastrokine 1 ELISA has not been compared to any commercial 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 manual washing
- 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

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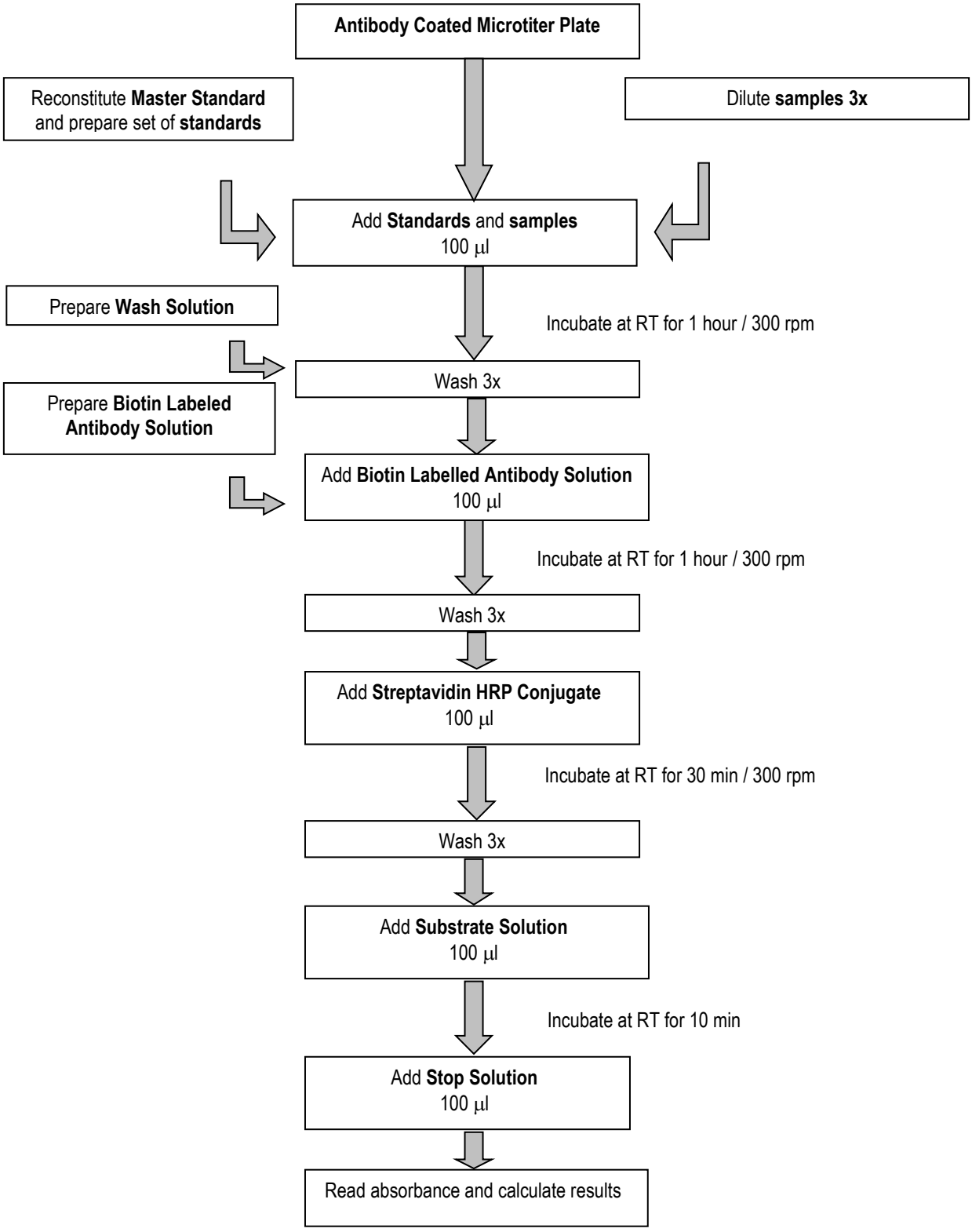
» **For more references on this product see our WebPages at www.biovendor.com**

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	Attention, see instructions for use
	Potential biological hazard
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

Assay Procedure Summary



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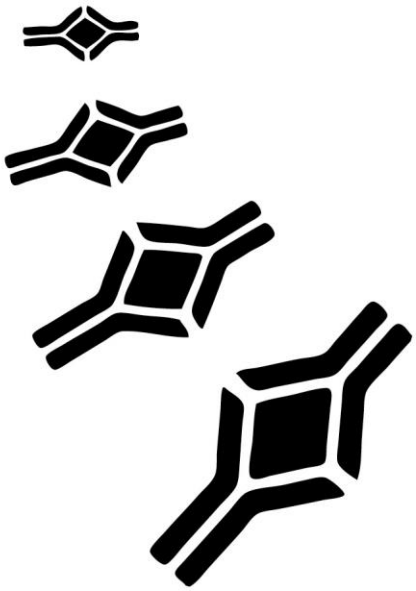
NOTES

NOTES



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