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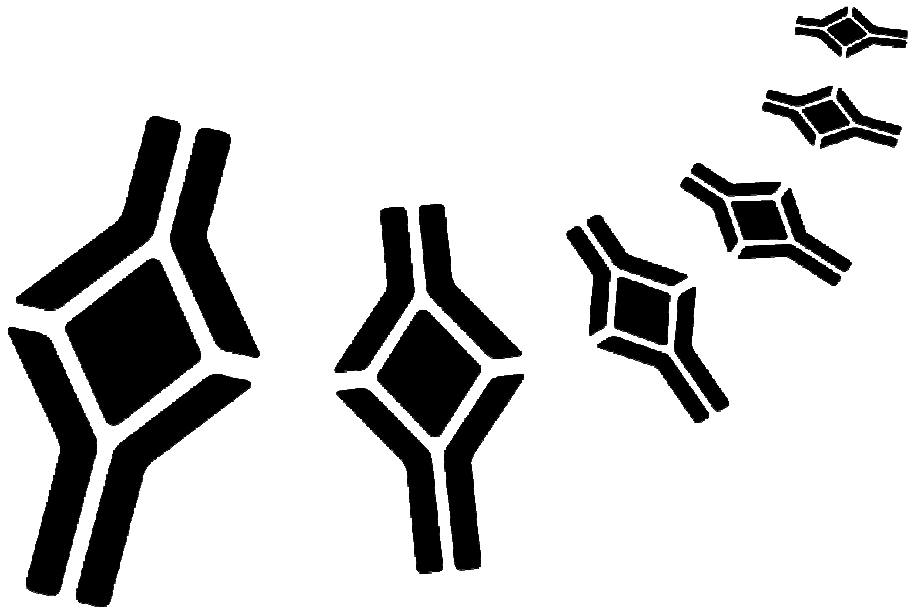
Research
and Diagnostic Products



HUMAN ZYMOGEN GRANULE MEMBRANE PROTEIN 16 ELISA

Product Data Sheet

Cat. No.: RD191229100R

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 RD191229100R Human Zymogen Granule Membrane Protein 16 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human zymogen granule membrane protein 16 (ZG16).

Features

- **It is intended for research use only**
- The total assay time is less than 3 hours
- The kit measures total human ZG16 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

Zymogen granule membrane protein 16 (ZG16) is a 16 kDa protein first identified by immunoscreening of a rat pancreatic cDNA expression library with a polyspecific antiserum raised against purified zymogen granule membranes (ZGM). ZG16 displays sequence homology especially in the carbohydrate recognition domain to the plant lectin jacalin, which recognizes terminal galactose attached to *N*-acetylgalactosamine by a β1-3 linkage. According to its sequence homology with this lectin, ZG16 was considered a secretory lectin ZG16. Sequence analyses uncovered that ZG16 is highly conserved amongst mammals and is also present in many other species.

Rat ZG16 was found to be highly expressed in the pancreas where the protein is localized in the zymogen granule of pancreas, colon, and duodenum. Previous reports indicated that rat ZG16 took part in the formation of zymogen granule by mediating the digestive enzymes to the zymogen granule membrane in pancreatic acinar cells.

Human ZG16 was shown to be highly expressed in the adult liver and moderately expressed in intestine (jejunum, ileum) and colon. Moreover, ZG16 is also weakly expressed in the pedunculus cerebellaris but not in other brain's regions.

Owing to the specific expression pattern in the liver, ZG16 was evaluated in hepatocellular carcinoma (HCC), a common cancer worldwide. It was found that human ZG16 was significantly down-regulated in HCC. ZG16 protein also plays a role in the secretion of several glycoproteins. The secretion of human ZG16 was affected when the synthesis of glycans was inhibited with either an inhibitor or by the lack of glucose in cell culture.

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

4. TEST PRINCIPLE

In the BioVendor Human Zymogen Granule Membrane Protein 16 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human ZG16 antibody. After 60 minutes incubation and washing, polyclonal anti-human ZG16 antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with the captured ZG16 protein. Following another washing step, the remaining HRP 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 spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of ZG16. 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
- The kit is intended to determine ZG16 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
Conjugate Solution Conc. (50x)	concentrated	0.26 ml
Conjugate Diluent	ready to use	13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer Conc. (10x)	concentrated	10 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 2–1000 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.

Conjugate Diluent
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:**

Dilution Buffer Conc. (10x)
Dilute Dilution Buffer Concentrate (10x) ten-fold in 90 ml distilled water to prepare a 1x working solution, e.g. 10 ml of Dilution Buffer Concentrate (10x) + 90 ml of distilled water for use of all 96-wells.
It is recommended to dilute only such a volume of Dilution Buffer Concentrate (10x) to be used up in the one run of the test.
Stability and storage:
The diluted Dilution Buffer is stable 1 week when stored at 2–8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2–8°C.

Human ZG16 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 ZG16 protein in the stock solution is **30 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	30 ng/ml
500 µl of stock	250 µl	20 ng/ml
360 µl of 20 ng/ml	360 µl	10 ng/ml
360 µl of 10 ng/ml	360 µl	5 ng/ml
360 µl of 5 ng/ml	240 µl	3 ng/ml
250 µl of 3 ng/ml	250 µl	1.5 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
The reconstituted standard stock solution must be used immediately.
Do not store the standard stock solution and set of standards.

Conjugate Solution Conc. (50x)
Prepare the working Conjugate Solution by adding 1 part Conjugate Solution Concentrate (50x) with 49 parts Conjugate Diluent.
Example: 0.25 ml of Conjugate Solution Concentrate (50x) + 12.25 ml of Conjugate Diluent for use of all 96-wells. Prepare only the volume needed for the test. **Mix well** (not to foam).
Stability and storage:
Opened Conjugate Solution Concentrate (50x) is stable 3 months when stored at 2–8°C.
Do not store the diluted Conjugate 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 ZG16 protein in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly 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 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°C, or preferably at -70°C for long-term storage.
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, Blank (Dilution Buffer) 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 **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 Conjugate Solution into each well.
5. Incubate the plate 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 Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **15 minutes** at room temperature. The incubation time may be extended, if the reaction temperature is below than 20°C. Do not shake with the plate during the incubation.
9. Stop the colour development by adding 100 µl of Stop Solution.
10. 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 9.**

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 ZG16 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 30**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 20**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 10**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 3**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 1.5**	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 ZG16 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 they have been diluted prior to the assay, e.g. 8 ng/ml (from standard curve) x 5 (dilution factor) = 40 ng/ml.

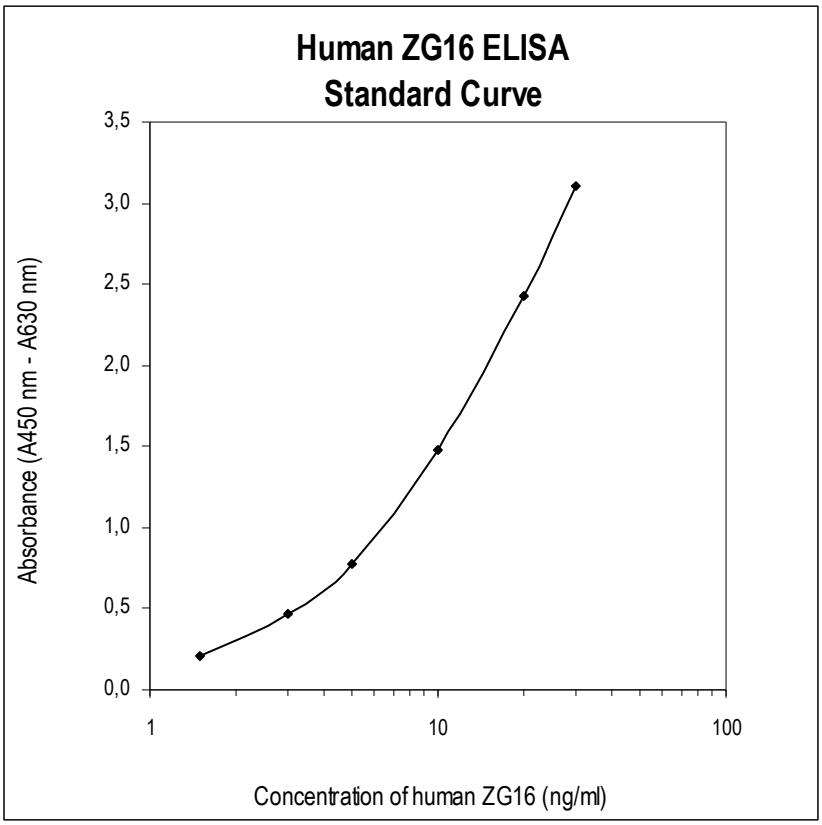


Figure 2: Typical Standard Curve for Human Zymogen Granule Membrane Protein 16 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Zymogen Granule Membrane Protein 16 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 human ZG16 values in wells and is 0.101 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**

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 ZG16 protein.

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	yes
Mouse	yes
Pig	no
Rabbit	no
Rat	no
Sheep	no

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	13.58	0.63	4.66
2	57.74	3.92	6.79

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	14.62	0.77	5.24
2	62.45	3.60	5.76

- **Spiking Recovery**

Serum samples were spiked with different amounts of human ZG16 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	13.25	-	-
	24.01	23.25	103.3
	34.35	33.25	103.3
	55.40	53.25	104.0
2	22.60	-	-
	32.43	32.60	99.5
	42.99	42.60	100.9
	66.45	62.60	106.2

- **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	-	39.95	-	-
	2x	19.83	19.98	99.3
	4x	10.83	9.99	108.4
	8x	5.19	4.99	103.8
2	-	46.47	-	-
	2x	24.13	23.23	103.8
	4x	13.09	11.62	112.7
	8x	6.29	5.81	108.3

- **Effect of sample matrix**

EDTA, citrate and heparin 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	45.44	43.34	42.70	43.54
2	53.36	53.08	51.32	57.18
3	47.76	49.42	45.68	45.84
4	28.71	33.77	29.02	30.39
5	40.40	43.55	40.12	40.40
6	38.86	39.16	35.77	38.75
7	31.78	41.53	31.01	39.35
8	37.39	43.39	37.07	42.23
9	45.04	51.30	43.74	47.44
10	57.08	55.82	53.11	59.61
Mean (ng/ml)	**42.58**	**45.44**	**40.95**	**44.47**
Mean Plasma/Serum (%)		**106.7**	**96.2**	**104.4**
Coefficient of determination R^2		**0.84**	**0.99**	**0.89**

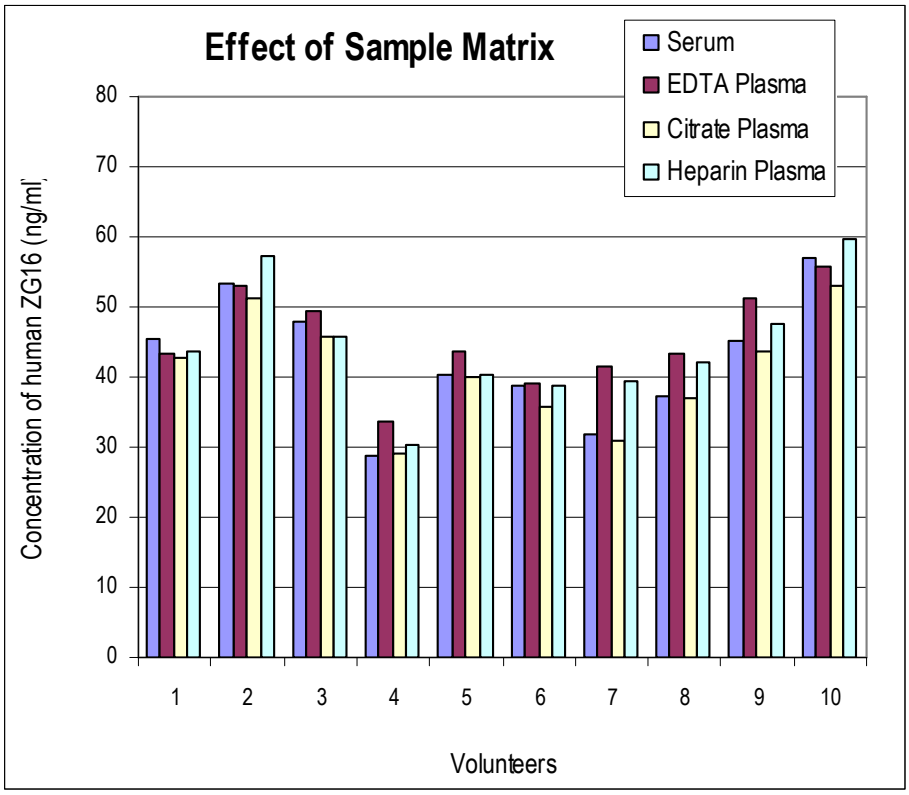


Figure 3: ZG16 levels measured using Human Zymogen Granule Membrane Protein 16 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2–8°C**

Samples should be stored at -80°C. However, no decline in concentration of ZG16 was observed in serum and plasma samples after 7 days when stored at 2–8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	*Storage conditions*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-80°C	34.99	40.29	30.98	31.94
	2–8°C, 1 day	32.16	34.87	29.67	29.15
	2–8°C, 7 days	34.89	37.15	30.21	28.34
2	-80°C	27.91	28.93	25.15	28.46
	2–8°C, 1 day	26.72	27.21	24.55	27.13
	2–8°C, 7 days	27.82	29.34	24.80	26.99
3	-80°C	37.51	36.09	33.52	35.26
	2–8°C, 1 day	34.25	38.66	31.91	33.91
	2–8°C, 7 days	36.03	38.05	32.56	33.59

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human ZG16 in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	*Number of f/t cycles*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	1x	43.70	49.13	39.75	39.03
	3x	42.98	48.24	38.76	39.87
	5x	44.60	50.04	36.87	38.83
2	1x	30.27	32.56	25.30	24.71
	3x	29.64	30.35	25.76	23.39
	5x	29.21	32.04	24.25	22.93
3	1x	39.84	41.76	36.13	33.95
	3x	39.17	44.32	38.18	32.97
	5x	36.88	43.47	36.78	34.47

14. DEFINITION OF THE STANDARD

In this assay a recombinant protein is used as the standard. The recombinant ZG16 is a 17.9 kDa protein consisting of 151 amino acid residues of the human ZG16 and 10 extra aminoacides.

15. REFERENCE RANGE

The reference range of serum samples from healthy volunteers (N=155) has been determined using this Human Zymogen Granule Membrane Protein 16 ELISA kit in our laboratory:
Mean concentration of human ZG16 from this healthy population was 45.2 ng/ml (SD=13.2).

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 ZG16 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

References to human ZG16:

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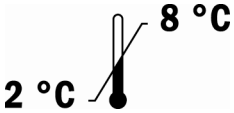
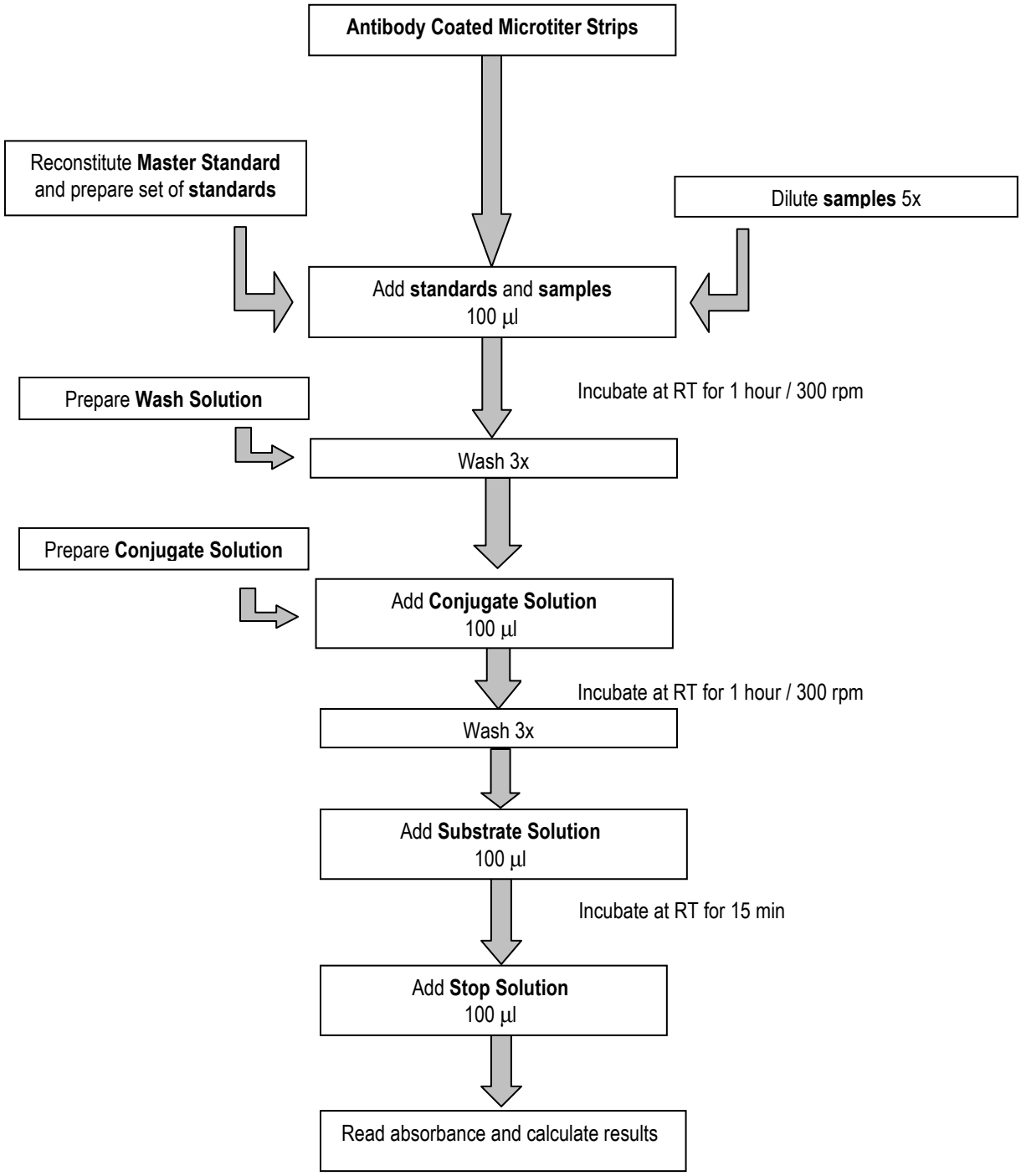
- Kleene R, Dartsch H, Kern HF. The secretory lectin ZG16p mediates sorting of enzyme proteins to the zymogen granule membrane in pancreatic acinar cells. *Eur J Cell Biol*; **78**(2):79-90 (1999)

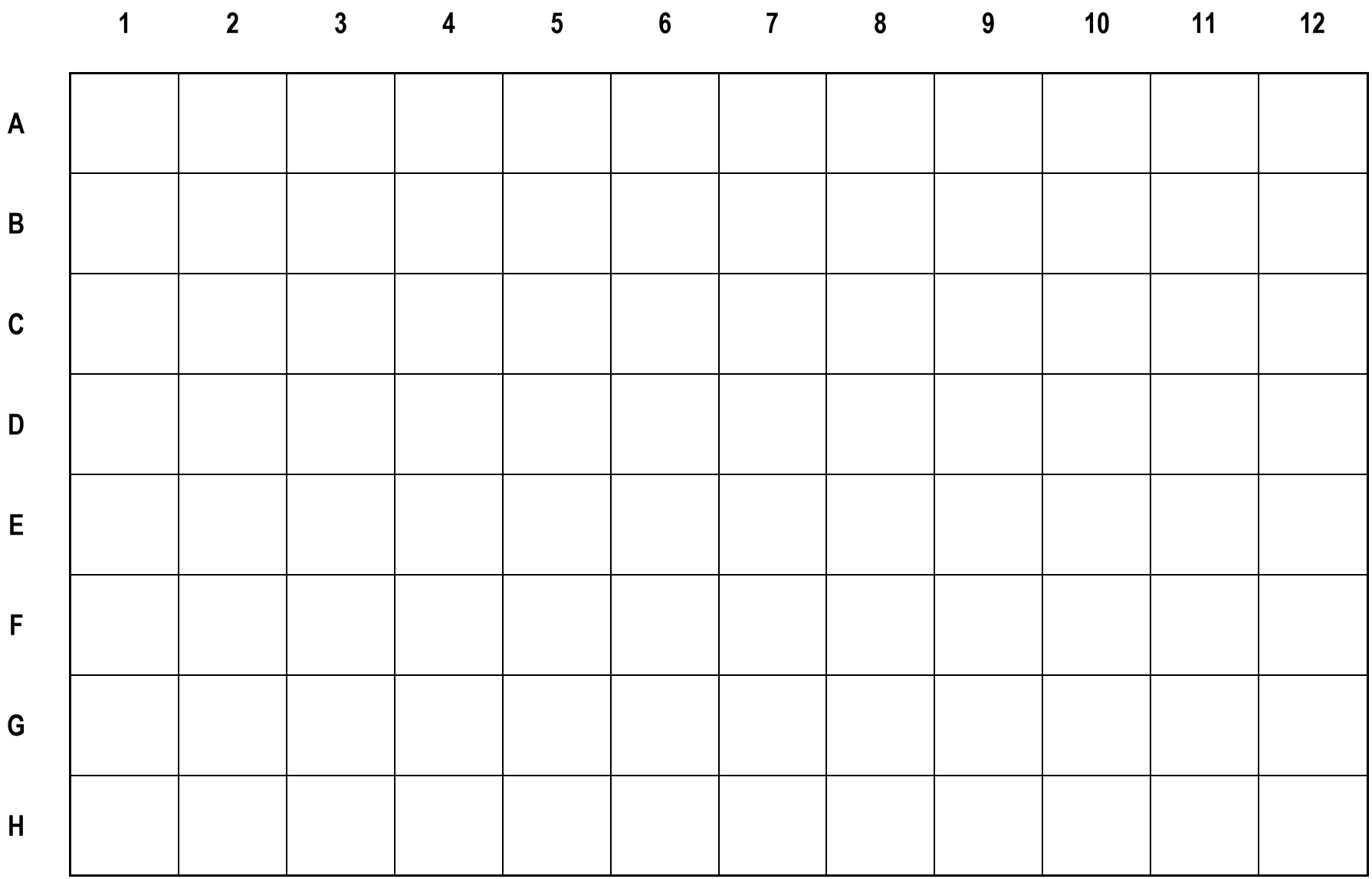
For more references on this product see our WebPages at www.biovendor.com

18. EXPLANATION OF SYMBOLS

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

Assay Procedure Summary

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NOTES

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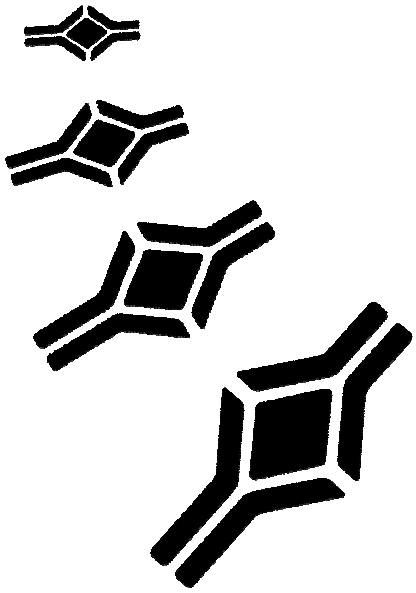

HEADQUARTERS: **BioVendor - Laboratorní medicína a.s.**	**Karásek 1767/1**	**621 00 Brno** **CZECH REPUBLIC**	**Phone: +420-549-124-185** **Fax: +420-549-211-460**	**E-mail: info@biovendor.com** **sales@biovendor.com** **Web: www.biovendor.com**
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	128 Bingham Rd. Suite 1300	Asheville, NC 28806 USA	Phone: +1-828-575-9250 +1-800-404-7807 Fax: +1-828-575-9251	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2917, 29/F R & F Ying Feng Plaza, No.2 Huaqiang road	Pearl River New Town Guang Zhou, CHINA	Phone: +86-20-38065519 Fax: +86-20-38065529	E-mail: infoCN@biovendor.com