BioVendor Group

ENG

Instructions for Use:

MOUSE/RAT GROWTH HORMONE (GH) ELISA

Catalogue number:

RMEE023R

For research use only.

BioVendor R&D

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.002.A	ENG.003.A
Format modifications in the document.	
Added symbol indicating manufacturer.	

1. INTENDED USE

The Mouse/Rat Growth Hormone (GH) ELISA RMEE023R is intended to be used for the measurement of Growth Hormone in mouse and rat serum and plasma samples for research use.

- For research use only!
- Enzyme-linked Immunoasssay
- Incubation period: 3 hours
- Antibodies: specific guinea pig and goat anti-mouse/rat-GH Antibodies
- Buffer: Ready for use and 20fold concentrate
- Standard: 7 Single standards: (0.15 – 9.0 ng/ml), recombinant Rat GH
- Assay Range: 0.04 – 45 ng/ml
- Control: 2 Control sera, lyophilized
- Sample: Mouse- and Rat-Serum /Plasma
- Required sample volume for 1:5 Dilution: 20 µl net for a single determination
- Sample dilution: Dependent on available sample volume: preferentially recommended
- 1:5 from 1:2,5 up to 1:30 (for high m/r GH levels)
- Analytical Sensitivity: Ø < 0.04 µg/l
- Intra/Interassay Variance: Ø < 5% / Ø < 10%

2. ASSAY PRINCIPLE

The BioVendor Mouse/Rat GH ELISA, RMEE023R is a so-called sandwich-assay. It utilizes two different specific high affinity polyclonal antibodies for this protein. The GH in the samples binds quantitatively to the immobilized antibody. In the following step, the biotinylated antibody in turn binds GH. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antibody. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the GH content of the sample. The reaction is stopped by the addition of stop solution and color intensity is quantified by measuring the absorption.

3. REAGENTS PROVIDED

The reagents listed below are sufficient for 96 wells including the standard curve

MTP	**Microtiter plate,** ready for use, coated with goat-anti-mouse/rat-GH antibodies, wells are separately breakable.	(8x12) wells
A-G	**Standards,** lyophilised (recombinant rat GH), Concentrations are given on the vial labels and quality certificate.	7 x 1 ml
KS1	**Control Serum 1**, lyophilised, (Rat Serum), Concentration is given **on the quality certificate.**	1x 150 µl
KS2	**Control Serum 2**, lyophilised, (Rat Serum), Concentration is given **on the quality certificate.**	1x 150 µl
AK	**Antibody Conjugate**, ready for use, guinea pig anti-m/r-GH-Antibody, biotinylated.	1 x 12 ml
EK	**Enzyme Conjugate EK**, contains HRP (Horseradish-Peroxidase)-labeled Streptavidin.	1 x 12 ml
VP	**Dilution Buffer**, ready for use. **Please shake before use.**	1 x 50 ml
WP	**Washing Buffer WP**, 20fold concentrated solution	1 x 50 ml
S	**Substrate S**, ready for use, horseradish-peroxidase (HRP)-substrate, stabilised Tetramethylbencidine.	1 x 12 ml
SL	**Stopping Solution SL**, ready for use, 0.2M sulphuric acid.	1 x 12 ml
-	**Sealing Tape for covering the microtiter plate**	3 x

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer WP (A. dest.), 950 ml
- Graduated cylinder for diluting Washing Buffer WP
- Vortex-mixer
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)

5. WARNINGS AND PRECAUTIONS

For research use only. For Professional use only.
The BioVendor kit is suitable only for in vitro and not for internal use in humans and animals. Follow strictly the test protocol. Be sure that everything has been understood. BioVendor will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.

Do not use obvious damaged or microbial contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.
Animal serum: mouse / rat in the following components: KS1, KS2

5.1 Reagents AK, EK, VP, WP

Contain as preservative 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

5.2 Substrate Solution (S)

The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbencidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+ P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

5.3 Stopping Solution (SL)

The Stopping solution contains 0.2 M acid sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician

5.4 General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.
Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.
Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

6. SAMPLE

6.1 Sample Type

Mouse-/Rat-Serum/Plasma
EDTA-plasma samples of rats were found to be increased by plus 100% in a comparative study, relating to rat serum GH-values (Lit. 1).

6.2 Sample Collection

Haemolytic conditions have to be avoided

6.3 Requested Sample Volume

20 µl net per single determination

6.4 Sample Stability

- Sample transport is recommended chilled e.g. on cooling elements (blue ice) or frozen on dry ice.
- in firmly closable sample vials
- Storage at -20°C: min. 2 years
- Freeze/-thaw cycles: max. 10

It is recommended to store samples chilled as soon as possible.
For any longer time storage the sample **has to be kept frozen at -20°C**.

6.5 Sample Dilution

For commercial pooled rodent sera a **1:5 dilution was found suitable**.
An extraction step is not required.

- Dilution: with Dilution Buffer **VP**:
- For a double determination: e.g. **50 µl** sample plus **200 µl** Dilution Buffer VP
- After mixing use **100 µl** diluted sample per well in the assay within **1 hour** of this solution.
- Where required, depending on the expected GH-values, the dilution with **Dilution Buffer VP** can be higher or lower.

Depending upon the used strain of the animals or the experimental conditions**, the endogenous content of GH can vary strongly**. It is recommended to test in advance the individual optimal sample pre-dilution under the respective conditions.

7. TECHNICAL RECOMMENDATIONS

7.1 Storage Conditions

Store the kit at 2-8°C after receipt until its expiry date.

7.2 Storage Life

The shelf life of the components **after initial opening** is warranted for **4 weeks**, store the unused strips and microtiter wells **airtight** together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The 1:20 diluted Washing Buffer **WP** is 4 weeks stable at 2-8°C. The **reconstituted components** standards **A-G** and Control Sera **KS1 and KS2** must be stored at -20°C (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Repeated freeze/thaw cycles have to be avoided, up to three cycles were found to have no influence.

7.3 Preparation of reagents

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming. Reagents with different lot numbers cannot be mixed.

7.4 Reconstitution

The Standards **A – G** and Control **KS1 and KS2** are reconstituted with the Dilution Buffer **VP**. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer. **Attention:** Standards should be thawed only once – where required please store aliquoted in adequate volumes.

7.5 Dilution

After reconstitution dilute the Control Sera **KS1 and KS2** with the Dilution Buffer **VP** in the same ratio e.g. (1:5) as the sample.
The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest.

7.6 Incubation

Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate Solution **S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive–store and incubation in the dark.

7.7 Assay Procedure

When performing the assay, Blank, Standards **A-G**, Control Serum **KS** and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate **AK,** Enzyme Conjugate **EK** as well as the succeeding Substrate Solution **S** should be added to the plate in the same order and in the same time interval as the samples. Stopping Solution **SL** should be added to the plate in the same order as Substrate Solution **S**.
All determinations (Blank, Standards **A-G**, Control Sera **KS1 and KS2** and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

7.8 Shaking

The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must be adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/ or false values, excessive shaking may result in high optical densities and/ or false values. **Substrate S** Incubation without shaking.

7.9 Washing

Proper washing is of basic **importance** for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.
All washing must be performed with the provided Washing Buffer **WP** diluted to usage concentration. Washing volume per washing cycle and well must be 300 µl at least.
The danger of handling with potentially infectious material must be taken into account.
When using an **automatic microtiter** plate washer, the respective instructions fur use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters,

must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.
Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamical swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

8. ASSAY PROCEDURE

Preparation of reagents:		**Reconstitution:**	**Dilution:**
A-G	Standards	in 1 ml Dilution Buffer VP	-
KS1	Control Serum 1	in 150 µl Dilution Buffer VP	1:5 with Dilution Buffer VP
KS2	Control Serum 2	in 150 µl Dilution Buffer VP	1:5 with Dilution Buffer VP
WP	Washing Buffer	-	1:20 with Aqua dest.

Sample and Control Sera KS1 and KS2: dilute 1:5 with Dilution Buffer **VP**, **mix immediately, incubate max. 60 min**. **Use 100 µl** for each well in the assay.
Before assay procedure bring all reagents to room temperature **20-25°C.**

Assay Procedure in Double Determination:		
Pipette	**Reagents**	**Position**
100 µl	Dilution Buffer VP (Blank)	A1/A2
100 µl	Standard A (0.15 ng/ml)	B1/B2
100 µl	Standard B (0.45 ng/ml)	C1/C2
100 µl	Standard C (0.90 ng/ml)	D1/D2
100 µl	Standard D (1.8 ng/ml)	E1/E2
100 µl	Standard E (3.6 ng/ml)	F1/F2
100 µl	Standard F (6.0 ng/ml)	G1/G2
100 µl	Standard G (9.0 ng/ml)	H1/H2
100 µl	Control Serum KS1 (1:5 diluted)	A3/A4
100 µl	Control Serum KS2 (1:5 diluted)	B3/A4
100 µl	Sample (1:5 diluted)	in the rest of the wells according the requirements pipettieren
Cover the wells with the sealing tape.		
Sample Incubation: 1 h at 20-25°C, 350 rpm		

5x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl each Washing Buffer WP/ well.	In each well
100 µl	Antibody Conjugate AK	In each well
Cover the wells with the sealing tape.		
Incubation: 1 h at 20-25°C, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl each Washing Buffer WP/ well.	In each well
100 µl	Enzyme Conjugate EK	In each well
Cover the wells with the sealing tape.		
Incubation: 0.5 h at 20-25°C, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl each Washing Buffer WP/ well.	In each well
100 µl	Substrate Solution S	In each well
Substrat S Incubation: 0.5 h in the Dark at 20-25°C		
100 µl	Stopping Solution SL	In each well
Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.		

9. CALCULATION OF RESULTS

9.1 Establishing the standard curve

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.30, these of standard G should be above 1.0.

Samples, which yield higher absorbance values than Standard G are beyond the standard curve, for reliable determinations these samples should be tested anew with a higher dilution.

Standards are provided in the following GH-concentrations:

Standard	**A**	**B**	**C**	**D**	**E**	**F**	**G**
ng/ml	0.15	0.45	0.90	1.8	3.6	6.0	9.0

1. Calculate the **mean absorbance** value for the blank from the duplicated determination (well A1/A2).

2. Subtract the mean absorbance of the blank from the mean absorbance of all other values.

3. Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.

4. Recommendation: Calculation of the standard curve should be done by using a computer program. **A higher-grade polynomial,** or **four parametric logistic (4-PL) curve fit** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).

5. The m/rGH concentration of the diluted sample or the diluted control sera KS1&2 in ng/ml is calculated in this way, the m/rGH concentration of the **undiluted sample** and of KS1 & KS2 is calculated **by multiplication** with the respective dilution factor.

9.2 Example of Typical Standard Curve

Standard	Blank	A	B	C	D	E	F	G
ng/ml	0	0.15	0.45	0.90	1.8	3.6	6.0	9.0
OD (450-620 nm)	0.1356	0.182	0.272	0.4	0.634	1.121	1.732	2.408

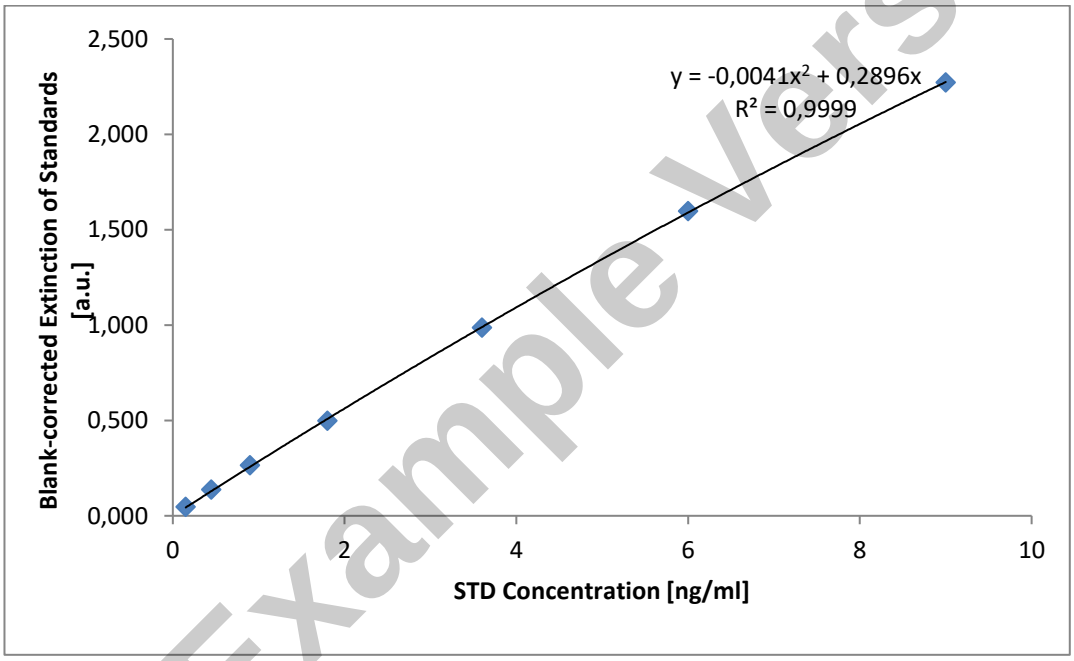


Figure 1 Exemplary Standard Curve

The exemplary shown standard curve in Fig.1 **cannot be used** for calculation of your test results. You have to establish a standard curve for each test you conduct!

Exemplary calculation of the GH concentration of a diluted sample:

OD 450 nm
Measured extinction (mean value) of your sample 1.5
Measured extinction of the blank (mean value) 0.1356
Your **measurement program** will calculate the m/rGH concentration of the sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit (here: polynomial 2nd degree).

In this exemplary case the following equation is solved by the program to calculate the GH concentration in the sample:

$$y = -0{,}0041x^2 + 0{,}2896x$$

$$5.09 = X$$

Multiplication by dilution factor (5) gives the GH concentration of the sample with **25.45** ng/ml.

10. PERFORMANCE CHARACTERISTICS

10.1 Calibration

The BioVendor RMEE023R was calibrated against a recombinant rat GH preparation.

10.2 Analytical Sensitivity

The analytical sensitivity of the ELISA RMEE023R was measured by the variability of the signal of the blank (by 15 to 16-fold determinations). Based on the twofold standard deviation of the blank the mean analytical sensitivity is < 0.04 ng/ml (Range 0.014 to 0.054 ng/ml).

10.3 Precision

The Inter- and Intra-Assay variation coefficients were on average <10% and <5%. Exemplary determinations are shown in table 1 and table 2.

	Mean Value (ng/ml)	Standard Deviation (ng/ml)	CV(%)
Sample 1	9.84	0.73	7.41
Sample 2	15.77	0.86	5.48

Table 1 Inter-Assay-Variation (n=7)

	Mean Value (ng/ml)	Standard Deviation (ng/ml)	CV (%)
Sample 1	10.03	0.32	3.22
Sample 2	3.74	0.17	4.55
Sample 3	16.16	0.33	2.01

Table 2 Intra-Assay-Variation (n=12)

10.4 Linearity

Linearity of sample dilution was tested by serial dilution of 3 rat sera. No diluted sample showed a relative standard deviation of >15 % in comparison to the respective mean rGH concentration. Linearity of sample dilution is shown by linear regression in the dilution range of 1:2.5 - 1:30 (Exemplary Sample 2 see Figure 2). We recommend preferentially a dilution of 1:5. Alternatively e.g. dilutions from 1:2.5 up to 1:30 (in case of higher rGH levels) dilutions would be suitable.

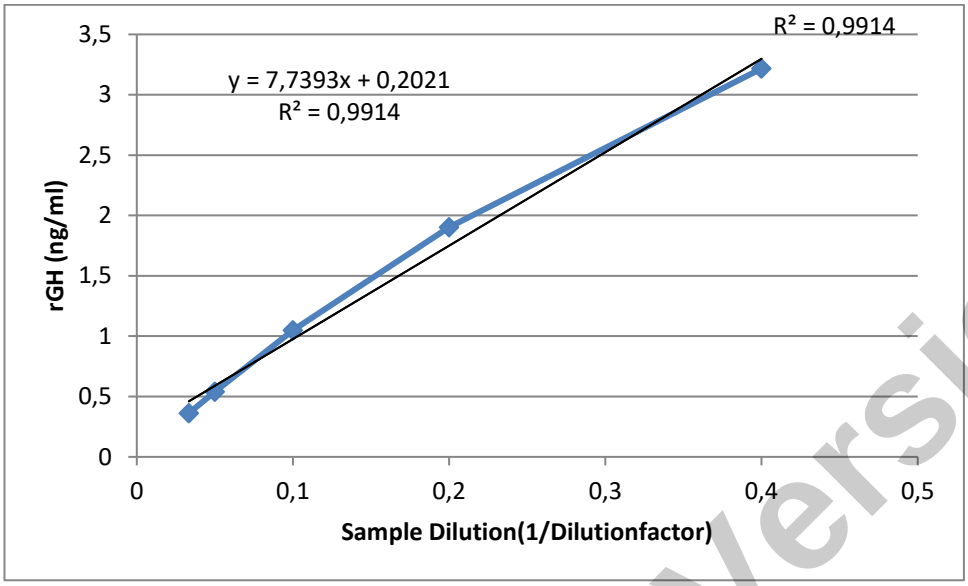


Figure 2 Exemplary regressions curve of the dilution (1:2.5 to1:30) of the rat sample.

Dilution:	Sample 1 (ng/ml)	Sample 2 (ng/ml)	Sample 2 (ng/ml)
1:2.5	15.1	8.0	3.968
1:5	17.7	9.5	4.62
1:10	19.6	10.5	5.329
1:20	20.8	10.8	5.508
1:30	21.3	10.8	
AV / SD / VC%	18.9 / 2.5 / 13.2	9.93 / 1.189 / 11.95	4.9 / 0.7 / 14.4

Rat serum samples were diluted in VP and rGH content was calculated. Measurements results are shown in [ng/ml]. No Coefficient of Variation >15 % was detected.
AV = Average Value, SD = Standard Deviation; VC = Coefficient of Variation

Table 3 Linearity

10.5 Interference

Interference of physiological appearing **Hemoglobin** with the m/rGH measurement was investigated. Serum samples have been enriched with different concentrations of possibly interfering **Hemoglobin** and the amount of m/rGH was measured and compared with the m/rGH concentration in the same sample without any enrichment. In Table the relative results are shown. **Hemoglobin did not** interfere significantly with m/rGH measurement.

	Hemoglobin 5 mg/ml
Sample 1	89
Sample 2	94
Sample 3	114

Table 4: Recovery [%] in comparison to the native serum.

10.6 Species Cross-Reactivity

Serum of the different species were used as diluted samples in this assay system. **No cross reactivity** was detected for:

Rabbit, Guinea pig, Dog, Cat, Chicken, Sheep, Goat, Pig, Donkey, Horse and Bovine.

No Cross reactivity was measured with recombinant human eukaryotic expressed GH (at 1 µg/ml)

11. REFERENCE

1. Popp S., Bielohuby M., Meurer S., Horngacher A., Bildingmaier M.; Abstract-Nr. P2 8-5-Analysis of different blood sample pre-treatment conditions on hormone concentrations in rats*; Quelle/ Source: Abstract-CD 55.* Symposium der Deutschen Gesellschaft für Endokrinologie 2012; ISSN 1862-1503

12. INTERNATIONAL TEST DESCRIPTION

A-G	STD	Rec in 1 ml BUF VP	-
KS1	Control	Rec in 150 µl BUF VP	1:5 DILU BUF VP
KS2	Control	Rec in 150 µl BUF VP	1:5 DILU BUF VP
WP	WASHBUF 20x	-	1:20 DILU A. dest.
SPE			1:5 DILU BUF VP
20-25 °C			
100 µl	BUF VP		A1/A2
100 µl	STD A (0.15 ng/ml)		B1/B2
100 µl	STD B (0.45 ng/ml)		C1/C2
100 µl	STD C (0.90 ng/ml)		D1/D2
100 µl	STD D (1.8 ng/ml)		E1/E2
100 µl	STD E (3.6 ng/ml)		F1/F2
100 µl	STD F (6.0 ng/ml)		G1/G2
100 µl	STD G (9.0 ng/ml)		H1/H2
100 µl	CONTROL KS 1 1:5 DILU BUF VP		A3/A4
100 µl	CONTROL KS 2 1:5 DILU BUF VP		B3/B4
100 µl	SPE 1:5 DILU BUF VP		
	TAPE		
1 h 20-25 °C ↔ 350 rpm			
5x 300 µl	5x WASHBUF WP		
100 µl	Ab AK		
	TAPE		
1 h 20-25°C ↔ 350 rpm			
5x 300 µl	5x WASHBUF WP		
100 µl	CONJ EK		
	TAPE		
0.5 h 20-25 °C ↔ 350 rpm			
5x 300 µl	5x WASHBUF WP		
100 µl	SUBST TMB S		
0.5 h 20-25 °C in the dark			
100 µl	H_2SO_4 SL		
	MEASURE		

13. ASSAY PROCEDURE - SUMMARY

Preparation of reagents:		Reconstitution:	Dilution:
A-G	Standards	in 1 ml Dilution Buffer VP	-
KS1	Control Serum 1	in 150 µl Dilution Buffer VP	1:5 with Dilution Buffer VP
KS2	Control Serum 2	in 150 µl Dilution Buffer VP	1:5 with Dilution Buffer VP
WP	Washing Buffer	-	1:20 with Aqua dest.

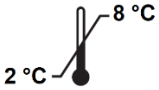
Sample and Control Sera KS1 and KS2: dilute 1:5 with Dilution Buffer **VP**, **mix immediately, incubate max. 60 min**. **Use 100 µl** for each well in the assay.

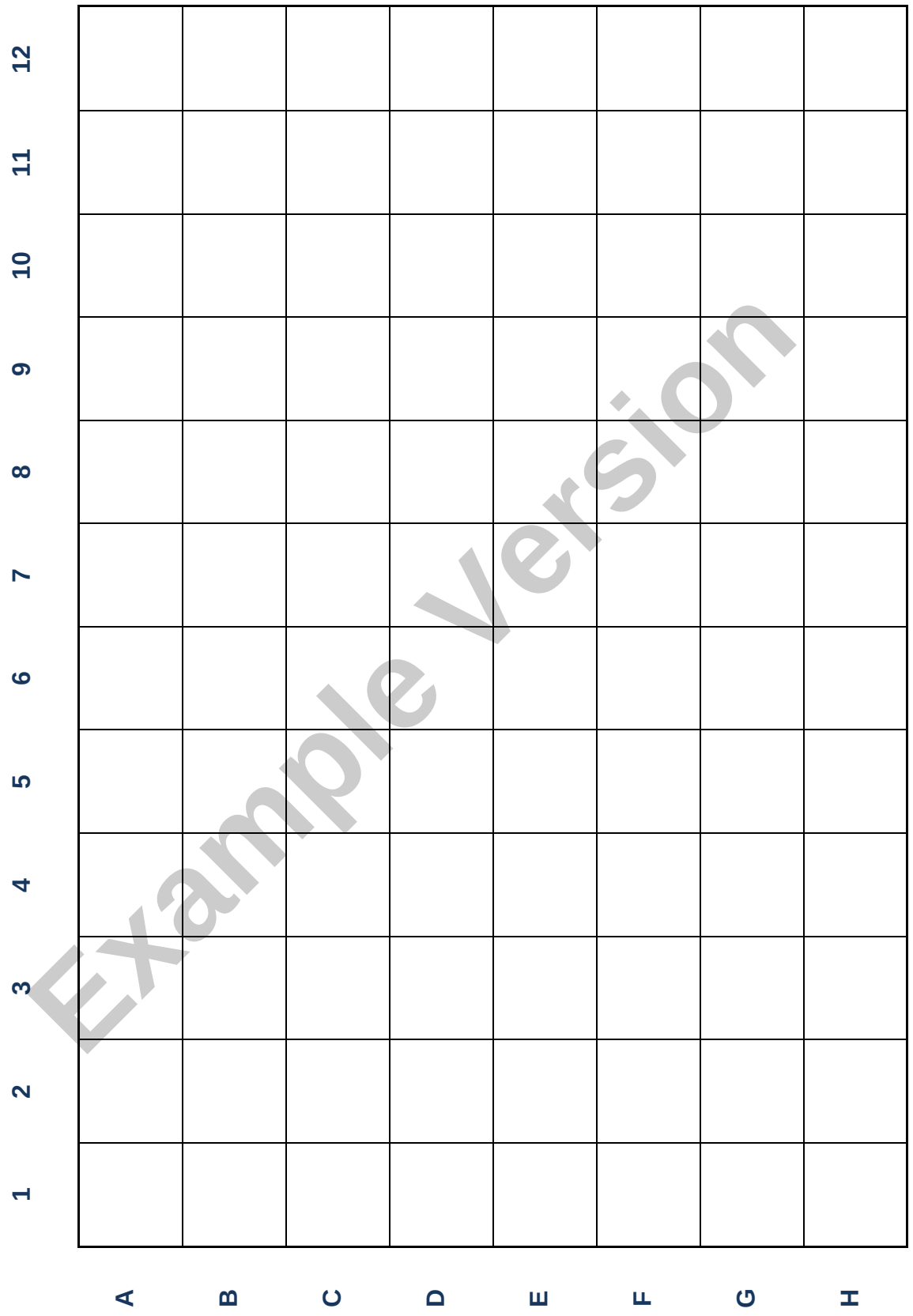
Before assay procedure bring all reagents to room temperature **20-25°C.**

Assay Procedure in Double Determination:		
Pipette	**Reagents**	**Position**
100 µl	Dilution Buffer VP (Blank)	A1/A2
100 µl	Standard A (0.15 ng/ml)	B1/B2
100 µl	Standard B (0.45 ng/ml)	C1/C2
100 µl	Standard C (0.90 ng/ml)	D1/D2
100 µl	Standard D (1.8 ng/ml)	E1/E2
100 µl	Standard E (3.6 ng/ml)	F1/F2
100 µl	Standard F (6.0 ng/ml)	G1/G2
100 µl	Standard G (9.0 ng/ml)	H1/H2
100 µl	Control Serum KS1 (1:5 diluted)	A3/A4
100 µl	Control Serum KS2 (1:5 diluted)	B3/A4
100 µl	Sample (1:5 diluted)	in the rest of the wells according to the requirements
Cover the wells with the sealing tape.		
Sample Incubation: 1 h at 20-25°C, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl each Washing Buffer **WP/ well**.	In each well
100 µl	Antibody Conjugate **AK**	In each well
Cover the wells with the sealing tape.		
Incubation: 1 h at 20-25°C, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl each Washing Buffer **WP/ well**.	In each well

100 µl	Enzyme Conjugate **EK**	In each well
Cover the recesses tightly with adhesive foil.		
Incubation: 0.5 h at 20-25°C, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl each Washing Buffer **WP/ well**.	In each well
100 µl	Substrate Solution **S**	In each well
Substrat S Incubation: 0.5 h in the Dark at 20-25°C		
100 µl	Stopping Solution **SL**	In each well
Measure the absorbance within **30 min** at **450 nm** with **≥ 590 nm** as reference wavelength.		

14. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks



BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com