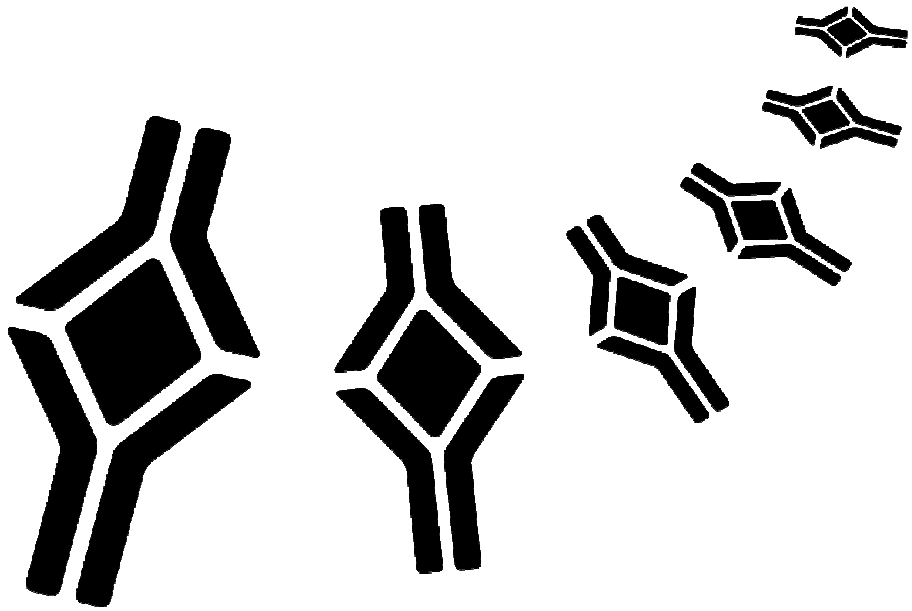
BioVendor
Research
and Diagnostic Products



HUMAN MYOSTATIN ELISA (Prodomain Specific)

Product Data Sheet

Cat. No.: RD191058200R

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 RD191058200R Human Myostatin ELISA (Prodomain Specific) is a sandwich enzyme immunoassay for the quantitative measurement of human Myostatin Latent Complex and free Myostatin Prodomain.

Features

- **It is intended for research use only**
- The assay time is less than 4 hours
- The kit measures total Myostatin Latent Complex and free Myostatin Prodomain in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- 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

Myostatin, also known as growth and differentiation factor 8 (GDF-8), belongs to the transforming growth factor β (TGF-β) superfamily of structurally related growth factors. Myostatin plays an essential role in regulating skeletal muscle growth and differentiation of tissues throughout the body and this function appears to be conserved across species [1].
Myostatin is expressed in human skeletal muscle as a biologically-inactive precursor protein (pre-promyostatin) consisting of an N-terminal signal peptide (23 amino acid residues), a propeptide domain (myostatin prodomain) which contains 243 amino acid residues, and a C-terminal domain containing 113 amino acids. Precursor protein (pre-promyostatin) forms a homodimer before proteolytic processing. After removal of the signal peptide, a furin protein convertase cleaves the promyostatin to generate myostatin propeptide and biologically active 12 kDa mature glycoprotein myostatin. The cleaved propeptide molecules remain non-covalently disulfide-linked to the mature C-terminal dimer forming the Latent Complex. [2,3,4].
The native myostatin circulates in serum as a part of the latent complex. Follistatin-related gene (FLRG) product has been reported to be an additional protein bound to circulating myostatin. The myostatin propeptide is known to bind and inhibit myostatin in vitro. This interaction is relevant in vivo, with a majority (>70%) of myostatin in serum being bound to its propeptide. Two molecules of myostatin propeptide inhibit the biological activity of one GDF-8 homodimer [5,6,7].
Overexpression of GDF-8 propeptide, follistatin or activin type II results in enhanced muscle development and increased skeletal muscle growth [8]. It has been shown that GDF-8 propeptide inhibits specific GDF-8 binding to L6 myoblast cells [9]. Myostatin mutation was found in a child with muscle hypertrophy which provides strong evidence that myostatin plays a significant role in regulating muscle mass in humans [10]. Myostatin promotes differentiation of multipotent mesenchymal cells into the adipogenic lineage and inhibits myogenesis. Studies have shown that myostatin could play an important role in cardiac development and physiology and has regulatory roles in fat metabolism [11,12]. Myostatin is expressed in myocardium where it exerts anti-hypertrophic, but profibrotic, effects. Circulating and local myostatin is elevated in chronic heart failure and is an important player in cardiac cachexia [13]. GDF-8 as a negative regulator of skeletal muscle mass has been implicated in several diseases involved in muscle wasting and cachexia. Circulating myostatin levels in male patients are elevated in chronic obstructive pulmonary disease (COPD) and the elevated myostatin levels are negatively correlated with total body skeletal muscle mass [14,15,16]. Myostatin not only regulates the growth of myocytes but also directly regulates muscle fibroblasts. Myostatin stimulates proliferation of muscle fibroblasts and production of extracellular matrix proteins both *in vitro* and *in vivo* [17]. GDF-8 itself or molecules that inhibit GDF-8 signalling may prove useful in the treatment of musculodegenerative states such as muscular dystrophy, neuromuscular diseases or cancer cachexia [18]. Myostatin blockade offers a strategy for reversing muscle wasting in Duchenne's muscular dystrophy (DMD) without resorting to genetic manipulation [8]. Latent TGFβ binding protein 4 (LTBP4) was identified as a genetic modifier of muscular dystrophy. It has been shown that myostatin forms a complex with LTBPand that overexpression of LTBP4 led to a decrease in myostatin levels. LTBP4 also

interacts with TGFβ and GDF11, a protein highly related to myostatin. These data identify LTBP4 as a multi-TGFβ family ligand binding protein with the capacity to modify muscle disease through overexpression [19].
Serum and intramuscular concentrations of myostatin-immunoreactive protein are increased in HIV-infected men with weight loss compared with healthy men and correlate inversely with fat-free mass index. It has been demonstrated that increased expression of the myostatin gene is associated with weight loss in men with AIDS wasting syndrome [20].

Areas of investigation:
Muscle growth and physiology regulation
Pulmonary diseases
Cardiovascular disease
Energy metabolism and body weight regulation

4. TEST PRINCIPLE

In the BioVendor Human Myostatin ELISA (Prodomain Specific), standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human myostatin prodomain antibody. After 120 minutes incubation and a washing, biotin-labelled polyclonal anti-human myostatin prodomain antibody is added and incubated 60 minutes. 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 total human Myostatin Latent Complex and free Myostatin Prodomain. A standard curve is constructed by plotting absorbance values against concentrations of Myostatin Prodomain 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 been 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
Dilution Buffer	ready to use	2 x 13 ml
Biotin-Ab Diluent	ready to use	13 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)
- Precise 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 shaking at 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.

Streptavidin-HRP Conjugate
Dilution Buffer
Biotin-Ab 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:**

Human Myostatin Prodomain 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 in the stock solution is **10 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	–	10 ng/ml
250 µl of stock	250 µl	5 ng/ml
250 µl of 5 ng/ml	250 µl	2.5 ng/ml
250 µl of 2.5 ng/ml	250 µl	1.25 ng/ml
250 µl of 1.25 ng/ml	250 µl	0.63 ng/ml
250 µl of 0.63 ng/ml	250 µl	0.31 ng/ml
250 µl of 0.31 ng/ml	250 µl	0.16 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

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 the reconstituted and/or diluted Biotin Labelled Antibody solutions.

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 Myostatin Latent Complex and free Myostatin Prodomain in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection, or should be stored frozen. 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.

An appropriate dilution should be assessed by the researcher in advance to batch measurement. Recommended starting dilution for serum and plasma is 20x.

Dilute serum samples 20x with Dilution Buffer just prior to the assay, e.g. 10 µl of sample + 190 µl of Dilution Buffer for singlets, or preferably 15 µl of sample + 285 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.

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of human Myostatin Latent Complex and free Myostatin Prodomain.

Note: It is recommended to use a precise 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 diluted 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 **2 hours**, shaking at ca. 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. 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 5-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 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 **15 minutes** at room temperature. The incubation time may be extended [up to 30 minutes] if the reaction temperature is less 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 human myostatin prodomain 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 four times. 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 10**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 2.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 1.25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 0.63**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 0.31**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 0.16**	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 Myostatin Latent Complex and free Myostatin Prodomain (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. 0.7 ng/ml (from standard curve) x 20 (dilution factor) = 14 ng/ml.

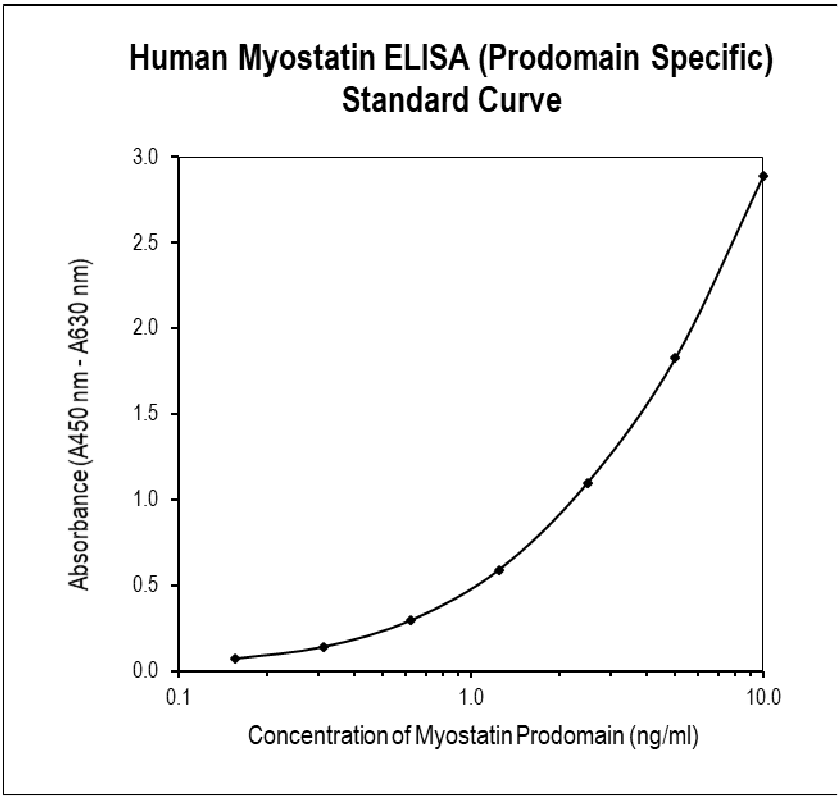


Figure 2: Typical Standard Curve for Human Myostatin ELISA (Prodomain Specific).

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Human Myostatin ELISA (Prodomain Specific) 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 Myostatin Latent Complex and free Myostatin Prodomain values in wells and is 0.05 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 Myostatin Latent Complex and free Myostatin Prodomain.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	26.71	0.96	3.6
2	83.76	1.13	1.3

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	15.07	1.36	9.1
2	25.58	2.50	9.8

- **Spiking Recovery**

Samples were spiked with different amounts of human recombinant Myostatin Prodomain and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	12.10	-	-
	18.22	18.30	99.2
	24.52	24.70	99.3
	35.00	37.10	94.3
2	21.18	-	-
	33.60	33.78	99.5
	45.78	46.18	99.3
	69.20	71.18	97.2

- **Linearity**

Samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml))*	*Recovery **O/E** (%)*
1	-	182.06	-	-
	2x	84.02	91.03	92.3
	4x	40.32	45.52	88.6
	8x	18.54	22.76	81.5
2	-	127.66	-	-
	2x	60.52	63.83	94.8
	4x	28.96	31.92	90.7
	8x	13.20	15.96	82.7

- **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	7.69	3.72	5.21	4.78
2	34.60	44.15	31.33	40.52
3	191.42	128.72	127.54	126.39
4	2.05	3.33	2.50	1.93
5	30.74	21.50	21.57	22.93
6	2.00	2.57	2.25	1.90
7	64.65	47.45	44.17	50.31
8	3.19	5.76	3.93	3.36
9	143.24	186.94	188.07	161.57
10	56.97	69.65	50.44	61.81
Mean (ng/ml)	**53.66**	**51.38**	**47.70**	**47.55**
Mean Plasma/Serum (%)	**-**	**95.8**	**88.9**	**88.6**
Coefficient of determination R^2	**-**	**0.83**	**0.84**	**0.89**

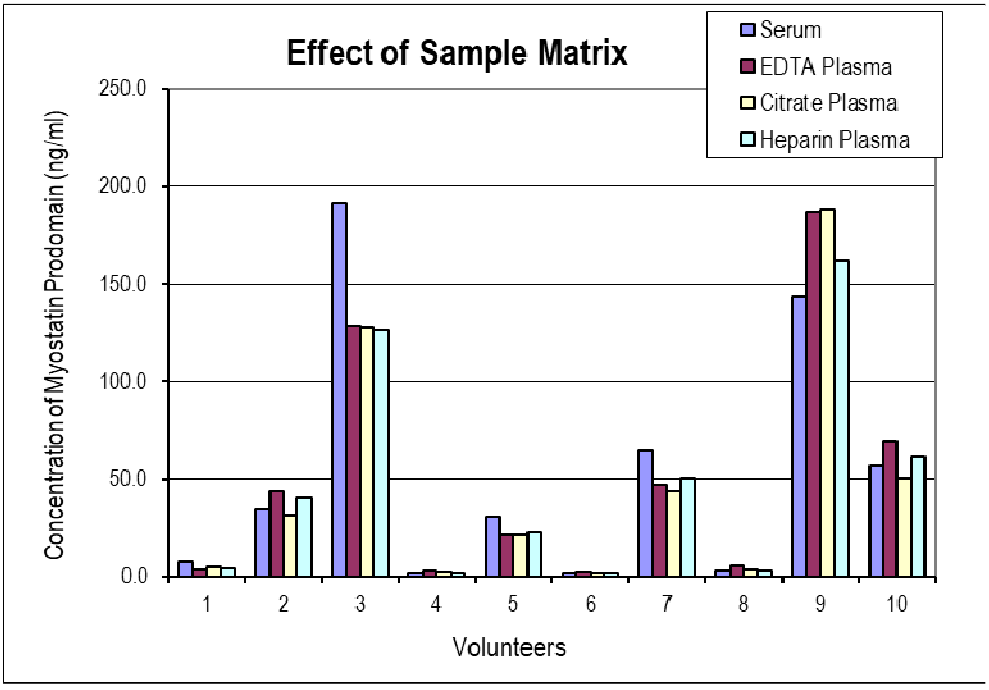


Figure 3: Myostatin Latent Complex and free Myostatin Prodomain levels measured using Human Myostatin ELISA (Prodomain Specific) in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is a recombinant protein produced in E. coli. It is the 28 kDa protein containing 243 amino acid residues (AA 24-266) of the human Myostatin Prodomain and 5 additional amino acid residues.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 21-65 years old were assayed with the BioVendor Human Myostatin ELISA (Prodomain Specific) in our laboratory.

- **Age dependent distribution**

Sex	*Age (years)*	*n*	*Myostatin Prodomain (ng/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	21-29	17	38.77	74.13	65.79	1.36	209.84
	30-39	25	55.29	25.95	61.68	1.36	224.66
	40-49	31	48.84	12.90	79.73	1.36	351.94
	50-65	16	54.15	3.72	111.95	1.43	457.23
Women	22-29	12	132.68	113.61	100.25	1.36	285.89
	30-39	26	35.75	7.63	50.50	1.43	199.63
	40-49	20	17.49	6.68	26.37	1.36	99.72
	50-61	8	72.39	2.45	2.45	1.36	221.30

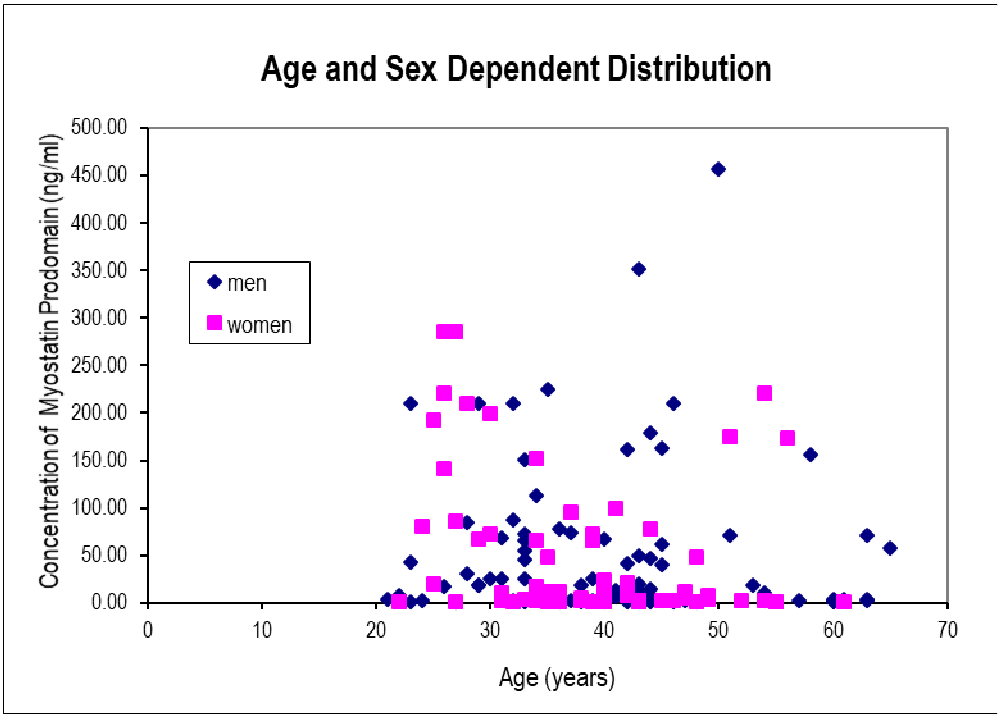


Figure 4: Human Myostatin Latent Complex and free Myostatin Prodomain concentration plotted against donor age and sex.

- **Reference range**

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 references ranges for Myostatin Latent Complex and free Myostatin Prodomain levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Myostatin ELISA (Prodomain Specific) has not been compared to any other 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 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 and samples

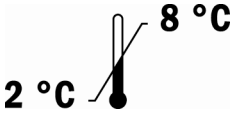
18. REFERENCES

1. McPherron AC, Lee SJ: Double muscling in cattle due to mutations in the myostatin gene. Proc Natl Acad Sci U S A. 1997; 94 (23):12457-61
2. Lee SJ: Regulation of muscle mass by myostatin. Annu rev Cell dev Biol 2004;20:61-86
3. Hill JJ, Davies MV, Pearson AA, Wang JH, Hewick RM, Wolfman NM, Qiu Y: The Myostatin Propeptide and the Follistatin-related Gene Are Inhibitory Proteins of Myostatin in Normal Serum. J Biol Chem. 2002;377(43):40735-40741
4. Zimmers TA, Davies MW, Koniaris LG, Haynes P, Esquela AF et al: Induction of cachexia in mice by systemically administered myostatin. Sciernce 2002;296:1486-88
5. Lee SJ, McPherron AC: Regulation of myostatin activity and muscle growth. Proc Natl Acad Sci U S A. 2001;98(16):9306-11
6. Diel P, Schiffer T, Geisler S, Hertrampf T, Mosler S, Schulz S, Wintgens KF, Adler M: Analysis of the effects of androgens and training on myostatin propeptide and follistatin concentrations in blood and skeletal muscle using highly sensitive Immuno PCR. Mol Cell Endocrinol. 2010;330(1-2):1-9
7. Jiang MS, Liang LF, Wang S, Ratovitski T, Holmstrom J, Barker C, Stotish R: Characterization and identification of the inhibitory domain of GDF-8 propeptide. Biochem Biophys Res Commun. 2004;315(3):525-31
8. Bogdanovich S, Perkins KJ, Krag TO, Whittemore LA, Khurana TS: Myostatin propeptide-mediated amelioration of dystrophic pathofysiology. FASEB J. 2005;19 (6):543-549
9. Thies RS, Chen T, Davies MV, Tomkinson KN, Pearson AA, Shakey QA, Wolfman NM: GDF-8 propeptide binds to GDF-8 and antagonizes biological activity by inhibiting GDF-8 receptor binding. Growth Factors. 2001;18(4):251-9
10. Schuelke M, Wagner KR, Stolz LE, Hübner C, Riebel T, Kömen W, Braun T, Tobin JF, Lee SJ: Myostatin Mutation Associated with Gross Muscle Hypertrophy in a Child. N Engl J Med. 2001;350(26):2682-2688
11. Sharma M, Kambadur R, Matthews KG, Somers WG, Devlin GP, Conaglen JV, Fowke PJ, Bass JJ: Myostatin, a transforming growth factor-beta superfamily member, is expressed in heart muscle and is upregulated in cardiomyocytes after infarct. J Cell Physiol. 1999;180(1):1-9
12. Artaza JN, Bhasin S, Magee TR, Reisz-Porszasz S, Shen R, Groome NP, Meerasahib MF, Gonzalez-Cadavid NF. Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. Endocrinology. 2005;146(8):3547-57
13. Dschietzig TB: Myostatin – from The Mighty Mouse to cardiovascular disease. Clin Chimica Acta 2014; 433:216-224
14. Taylor WE, Bhasin S, Artaza J, Byhower F, Azam M, Willard DH Jr, Kull FC Jr, Gonzalez-Cadavid N: Myostatin inhibits cell proliferation and protein synthesis in C2C12 muscle cells. Am J Physiol Endocrinol Metab. 2001;280(2):E221-8
15. Ju CR, Chen RC: Serum myostatin levels and skeletal muscle wasting in chronic obstructive pulmonary disease. Resp Med. 2012;106:102-108
16. Breitbart A, Scharf GM, Duncker D, Widera Ch,Gottlieb J, Vogel A, Schmidt S, Brandes G, Heuf HG, Lichtinghagen R, Kempf T, Wollert KC, Bauersachs J, Heinek J: Highly Specific

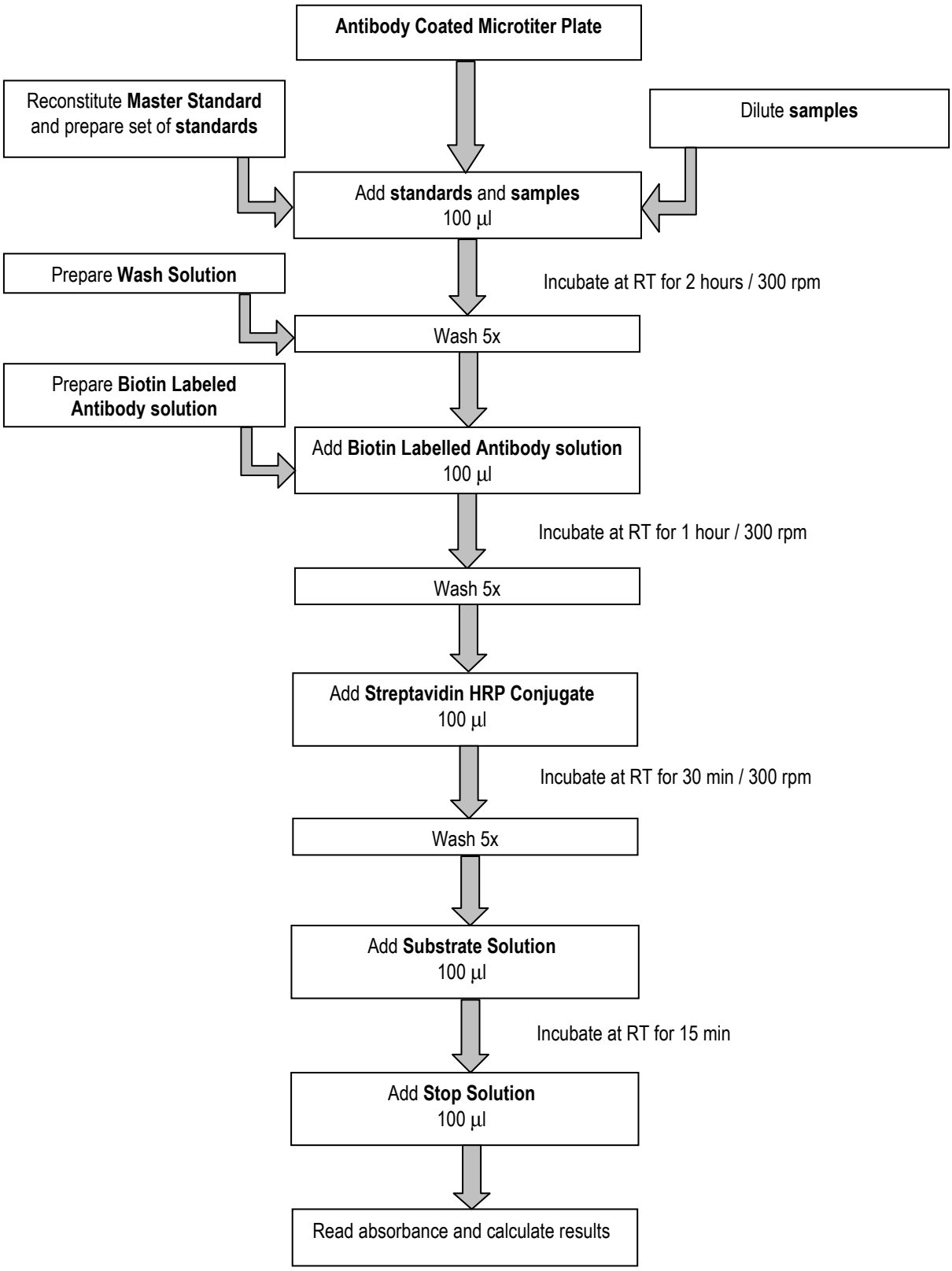
detection of Myostatin prodomain by an Immunoradiometric Sandwich Assay in Serum of Healthy Individuals and Patients. Plos One 2013;8(11):e80454
17. Li ZB, Kollias HD, Wagner KR: Myostatin Directly Regulates Skeletal Muscle Fibrosis. J Biol Chem 2008; 283(28): 19371–78
18. McPherron AC, Lawler AM, Lee SJ: Regulation OF skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature, 1997;387:83-90
19. Lamar KM, Bogdanovich S, Gardner BB, Gao QQ, Miller T, Earley JU, Hadhazy M, Wo AH, Wren L, Molkentin JD, McNally EM: Overexpression of Latent TGFβ Binding Protein 4 in Muscle Ameliorates Muscular Dystrophy through Myostatin and TGFβ. Plos genetics 2016;12(5):e1006019 (DOI:10.1371/journal.pgen.1006019)
20. Gonzalez-Cadavid NF, Taylor WE, Yarasheski K, Sinha-Hikim I, Ma K, Ezzat S, Shen R, Lalani R, Asa S, Mamita M, Nair G, Arver S, Bhasin S. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. Proc Natl Acad Sci U S A. 1998;95(25):14938-43

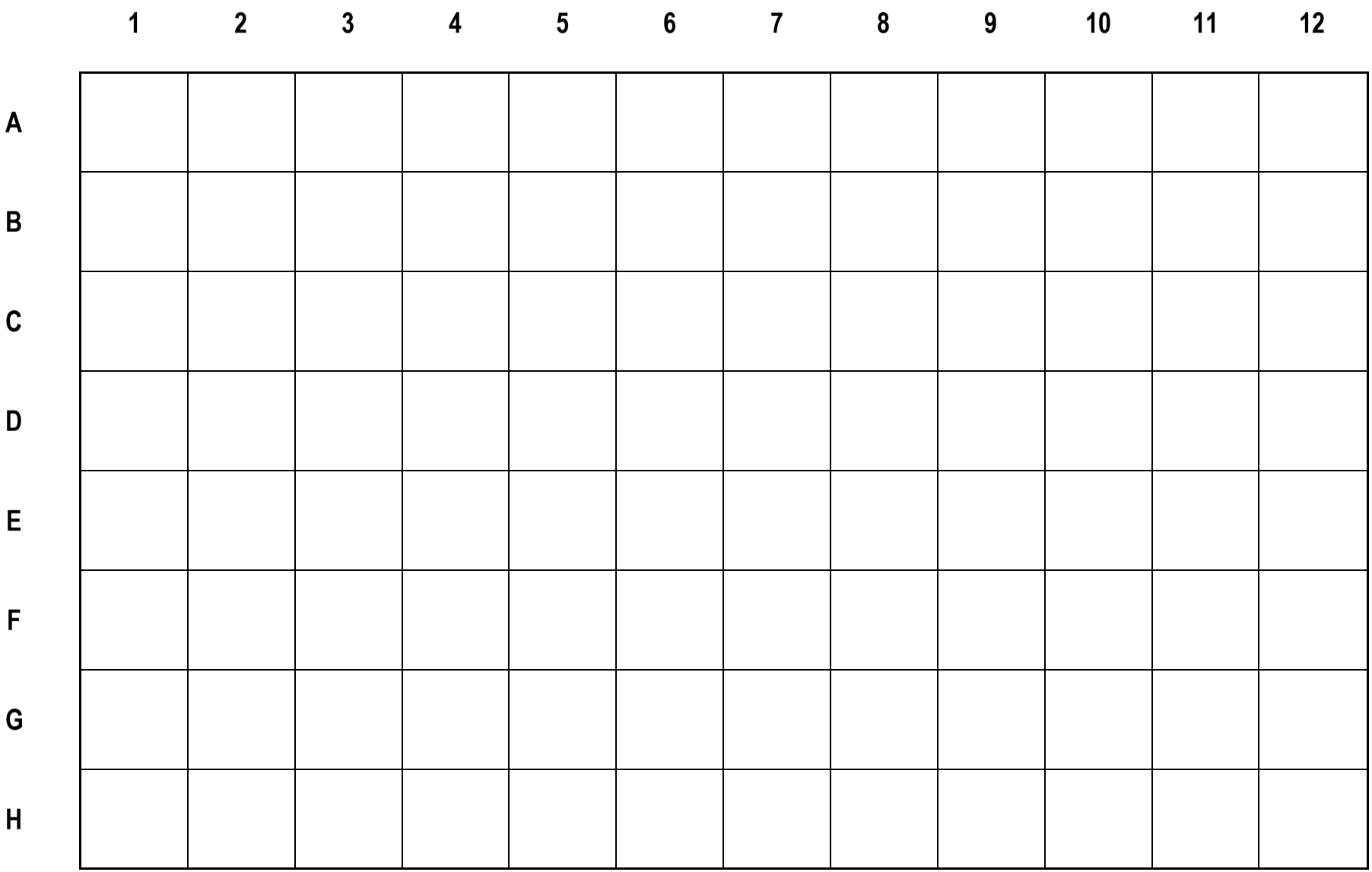
» **For more references on this product see our WebPages at www.biovendor.com**

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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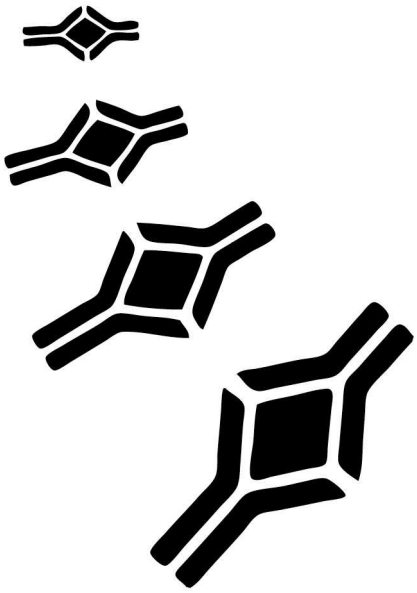

BioVendor – Laboratorní medicína a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420-549-124-185, Fax: +420-549-211-460
E-mail: info@biovendor.com, sales@biovendor.com
Web: www.biovendor.com

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