Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

Manual

Myostatin ELISA

For the determination of Myostatin in human serum and plasma

Valid from 2019-01-21

REF **KR1012**

Σ 96

+8 °C
+2 °C

RUO

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1. INTENDED USE

This Immundiagnostik assay is an enzyme immunoassay intended for the quantitative determination of myostatin in human serum and plasma.

For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Myostatin belongs to the transforming growth differentiation factor-ß (TGF-ß) super family. The molecule is a negative regulator of muscle growth, but details about the actions of myostatin are uncertain (Roth and Walsh, 2004).

Myostatin was first identified in 1997 by McPherron et al. They found out that null-mutant knockout mice were significantly larger than wild-type animals and exhibited a large and widespread increase in skeletal muscle mass due to an increase of muscle fiber number (hyperplasia) and thickness (hypertrophy). Other groups identified mutations in the myostatin gene in naturally bred "double-muscles" cattle breeds.

Similar to the findings in animal models, increased myostatin immuno-reactivity or expression has been observed in HIV-infected men with muscle wasting (Gonzales-Cadavid et al. 1998), after prolonged bed rest in young men (Zachwieja et al. 1999) and in older men and women with muscle wasting (Yarasheski KE et al. 2002).

Shi et al. (2007) and others have found that myostatin deficiency inhibits adipogenesis *in vivo*, even when mice are fed a high-fat diet. Transgenic overexpression of myostatin pro-peptide, which inhibits myostatin signaling, also inhibits body fat gain with a high-fat diet (Zhao et al. 2005). Similar alterations in myostatin signaling are associated with changes in body fat among humans.

Hittel et al. (2010) report that myostatin-levels are regulated by aerobic exercise. Moreover, myostatin is in the causal pathway of acquired insulin resistance with physical inactivity.

Indications

- Regulation of muscle growth
- Muscle atrophy
- Muscle wasting
- Acquired insulin resistance

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR1012	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml
KR1012	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
KR1012	STD	Standards, lyophilised (see specification for concentrations)	2 x 5 vials
KR1012	TRACER	Tracer concentrate, biotinylated myostatin	1 x 150 µl
KR1012	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial
KR1012	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial
KR1012	CONJ	Conjugate concentrate, streptavidin-labelled peroxidase	1 x 200 µl
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- **Preparation of the tracer:** Before use, the **tracer concentrate (TRACER)** has to be diluted **1:101** in sample dilution buffer (100 µl TRACER + 10 ml SAMPLEBUF). The TRACER is stable at **2–8 °C** until the expiry date stated on the label. **Tracer** (1:101 diluted TRACER) **is not stable and cannot be stored.**
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) **are not stable and cannot be stored.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Store EDTA plasma and serum until use at -20°C.

Preparation of samples

1.	Pipet **20 µl** of each **serum or plasma** sample in the respective labelled 1.5 ml reaction tubes.
2.	Add **180 µl** of **sample dilution buffer** to each sample, mix well. This results in a **dilution factor of 1:10**
3.	Add **200 µl tracer** (diluted TRACER) to each **diluted sample**, mix well. The prepared sample is named **pre-incubate.**

Preparation of standards and controls

Transfer **200 µl standard or control** in the corresponding reaction tubes, add **200 µl tracer** (diluted TRACER), mix well. Each treated standard or control is also named **pre-incubate.**

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of myostatin in serum and EDTA-plasma.

The assay is based on the method of a competitive ELISA. As a first preparation step, a biotinylated myostatin tracer is added to the samples, standards and controls. Afterwards, aliquots of the treated preparations are transferred and incubated in microtiter plate wells coated with polyclonal anti-myostatin antibodies. During the incubation, the free target antigen in the samples competes with the biotinylated myostatin tracer for the binding of the polyclonal anti-myostatin antibodies immobilised on the microtiter plate wells. The unbound components are removed by a washing step. During a second incubation step, a streptavidin-labeled-peroxidase antibody, which binds to the biotinylated myostatin tracer, is added into each microtiter well. After a washing step to remove the unbound components, the peroxidase substrate tetramethylbenzidine is added. Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow. The intensity of the yel-

low colour is inverse proportional to the myostatin concentration in the sample; this means, high myostatin concentration in the sample reduces the concentration of the biotinylated myostatin tracer bound to the immobilised anti-myostatin antibodies and lowers the photometric signal. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Myostatin, present in the patient samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until the expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Add each **100 µl** of the pre-treated **standards/controls/samples** (corresponding pre-incubate) into the respective wells.
2.	Cover the strips and incubate for **2 hours** on a **horizontal shaker** at room temperature (15-30 °C).
3.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
4.	Add **100 µl conjugate** (diluted CONJ) into each well.
5.	Cover the strips and incubate for **1 hour** on a **horizontal shaker** at room temperature (15-30 °C).
6.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
7.	Add **100 µl TMB substrate** (SUB) into each well.
8.	Incubate for **10–20 minutes*** at room temperature (15–30 °C) in the **dark**.

9.	Add **100 µl ELISA stop solution** (STOP) and mix well.
10.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the lowest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum and plasma samples

The obtained results have to be multiplied with the **dilution factor of 10** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

9. LIMITATIONS

Whole blood is not suitable. Untreated lipemic samples may produce incorrect results.

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the calibration curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 23)

Sample	Myostatin [ng/ml]	CV [%]
1	24.1	10.4
2	37.9	7.8

Inter-Assay (n = 6)

Sample	Myostatin [ng/ml]	CV [%]
1	18.1	12.2
2	14.8	14.0

Analytical Sensitivity

The LoB (limit of blank) was evaluated according to the guideline CLSI EP17-A2 and resulted in 0.37 ng/ml.

Dilution recovery

Two patient serum samples were diluted and analysed. The results are shown below (n = 2):

Sample	Dilution	Myostatin expected [ng/ml]	Myostatin measured [ng/ml]
1	1:10		30.4
	1:20	15.2	12.5
	1:40	7.6	8.7
2	1:10		29.0
	1:20	14.5	13.9
	1:40	7.3	8.2

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still should be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any

spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

General literature

1. Dschietzig, T.B., 2014. Myostatin - From the Mighty Mouse to cardiovascular disease and cachexia. *Clinica chimica acta; international journal of clinical chemistry*, **433C**, pp.216–224.
2. Gonzalez-Cadavid, N.F. et al., 1998. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proceedings of the National Academy of Sciences of the United States of America,* **95**(25), pp.14938–43.
3. Hamrick, M.W. et al., 2006. Increased muscle mass with myostatin deficiency improves gains in bone strength with exercise. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*, **21**(3), pp.477–83.
4. McPherron, a C. & Lee, S.J., 1997. Double muscling in cattle due to mutations in the myostatin gene. *Proceedings of the National Academy of Sciences of the United States of America*, **94**(23), pp.12457–61.
5. Turner, C.H., 2000. Muscle-bone interactions, revisited. *Bone*, **27**(3), pp.339–40.
6. Yarasheski, K.E. et al., 2002. Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting. *The journal of nutrition, health & aging*, **6**(5), pp.343–8.
7. Zachwieja, J.J. et al., 1999. Plasma myostatin-immunoreactive protein is increased after prolonged bed rest with low-dose T3 administration. *Journal of gravitational physiology : a journal of the International Society for Gravitational Physiology*, **6**(2), pp.11–5.

Literature using Immundiagnostik Myostatin-ELISA

8. Bowser, M. et al., 2013. Effects of the activin A-myostatin-follistatin system on aging bone and muscle progenitor cells. *Experimental gerontology*, **48**(2), pp.290–7.
9. Cheung, W.W. et al., 2008. Modulation of melanocortin signaling ameliorates uremic cachexia. *Kidney international*, **74**(2), pp.180–6.
10. Diel, P. et al., 2010. Analysis of the effects of androgens and training on myostatin propeptide and follistatin concentrations in blood and skeletal muscle using highly sensitive immuno PCR. *Molecular and cellular endocrinology*, **330**(1-2), pp.1–9.

11. Ehehalt, S. et al., 2011. Investigation of myostatin serum levels before and after a 6-month lifestyle intervention program in obese children. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association*, **119**(4), pp.238–42.

12. Gruson, D. et al., 2011. Increased plasma myostatin in heart failure. *European journal of heart failure*, **13**(7), pp.734–6.

13. Hittel, D.S. et al., 2010. Myostatin decreases with aerobic exercise and associates with insulin resistance. *Medicine and science in sports and exercise*, **42**(11), pp.2023–9.

14. Kukreti, H. et al., 2013. Muscle-specific microRNA1 (miR1) targets heat shock protein 70 (HSP70) during dexamethasone-mediated atrophy. *The Journal of biological chemistry*, **288**(9), pp.6663–78.

15. Szulc, P. et al., 2012. Endocrine and clinical correlates of myostatin serum concentration in men--the STRAMBO study. *The Journal of clinical endocrinology and metabolism*, **97**(10), pp.3700–8.

16. Vamvini, M.T. et al., 2013. Irisin mRNA and circulating levels in relation to other myokines in healthy and morbidly obese humans. *European journal of endocrinology / European Federation of Endocrine Societies*, **169**(6), pp.829–34.

17. Wintgens, K.F. et al., 2012. Plasma myostatin measured by a competitive ELISA using a highly specific antiserum. *Clinica chimica acta; international journal of clinical chemistry*, **413**(15-16), pp.1288–94.

18. Zamora, E. et al., 2010. Serum myostatin levels in chronic heart failure. *Revista española de cardiología*, **63**(8), pp.992–6.

Used symbols:


Temperature limitation

Catalogue Number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet