Verwendete Symbole:


Temperaturbegrenzung

REF Bestellnummer

RUO Nur für Forschungszwecke


Inhalt ausreichend für <n> Prüfungen


Hersteller


Verwendbar bis

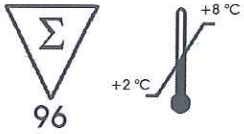
LOT Chargenbezeichnung

Myostatin ELISA Kit

For the determination of Myostatin in human serum and plasma

Valid from 13.02.2013

REF **K 1012**



RUO

1. INTENDED USE

This enzyme immunoassay is intended for the quantitative determination of myostatin in human serum and plasma. It is for research use only.

2. SUMMARY

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 immunoreactivity 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. PRINCIPLE OF THE TEST

This Enzyme-Linked-Immunosorbent Assay (ELISA) can be used 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 immobilized 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 (TMB) is added. Finally, the enzymatic reaction is terminated by an acidic stop solution. The color changes from blue to yellow and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow color 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 immobilized anti-myostatin antibodies and lowers the photometric signal. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standard.

4. MATERIAL SUPPLIED

Catalogue No	Content	Kit Components	Quantity
K 1012MTP	PLATE	One holder with precoated strips	12 x 8 wells
K 1012WP	WASHBUF	ELISA wash concentrate, 10x	2 x 100 ml
K 1012PV	SAMPLEBUF	Sample dilution buffer	1 x 100 ml
K 1012ST	STD	Standards (lyophilized)	2 x 6 vials
K 1012T	TRACER	Tracer, biotinylated myostatin	1 x 150 µl
K 1012KO1	CTRL	Control (lyophilized)	2 x 1 vial
K 1012KO2	CTRL	Control (lyophilized)	2 x 1 vial
K 1012K	CONJ	Conjugate (Streptavidin-labeled peroxidase)	1 x 200 µl
K 1012TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	1 x 15 ml
K 1012AC	STOP	ELISA stop solution, ready to use	1 x 15 ml

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Precision pipettors and disposable tips to deliver 10 - 1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge
- Vortex-Mixer
- Standard laboratory polypropylene reaction vessels (1.5 ml)
- Standard laboratory reaction vessel (15 ml)
- Microtiter plate reader at 450 nm

*Immundiagnostik AG recommends the use of Ultra Pure 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 0,055µS/cm at 25°C (≥18,2MΩ cm).

6. PREPARATION AND STORAGE OF REAGENTS

- Prior to use in the assay allow all reagents and samples to come to room temperature (18-26°C) and mix well.
- Reagents with a **volume less than 100 µl** should be centrifuged before use to avoid loss of volume.
- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted with ultra pure water **1:10** before use (e.g. 100 ml WASHBUF + 900 ml ultra pure water), mix well. For the diluted solution the term **wash buffer** is used. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be dissolved at 37°C using a water bath before dilution of the buffer solutions. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** could be stored in a closed flask at **2-8°C for one month**.
- The lyophilized **STD** (standards) and **CTRL** (controls) are stable at **2-8°C** until the expiry date stated on the label. (Volume and concentration, see product specification). **Reconstituted standards and controls are not stable.**
- The **TRACER** (biotinylated myostatin-tracer) must be diluted **1:101** in **SAMPLEBUF** (Sample dilution buffer) (e.g. 100 µl TRACER + 10 ml SAMPLEBUF). The **TRACER** (biotinylated myostatin-tracer) is stable at **2-8°C** until expiry date given on the label. **Diluted TRACER is not stable and cannot be stored.**
- The **CONJ** (Conjugate) must be diluted **1:101** in **wash buffer** (e.g. 100 µl CONJ + 10 ml wash buffer). The **CONJ** (Conjugate) is stable at **2-8°C** until expiry date given on the label. **Diluted CONJ is not stable and cannot be stored.**
- Take as many microtiter strips (PLATE) as needed from kit. Store unused microtiter strips sealed in the aluminium foil bag with desiccating agent at 2-8°C. Strips are stable until the expiry date stated on the label.
- All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at **2-8°C.**

7. SAMPLE PREPARATION AND STORAGE

Either EDTA plasma or serum can be used for analysis.

Store samples until use at -20°C.

Pipette **20 µl of each serum or plasma sample** in the respective labeled 1.5 ml reaction cups. Add **180 µl of SAMPLEBUF** (sample dilution buffer) to each sample, vortex well.

Dilution factor 1:10

Then add **200 µl diluted TRACER** to **200 µl of each diluted sample**, vortex well. The prepared sample is named **pre-incubate**.

8. STANDARD AND CONTROL PREPARATION

Transfer **200 µl of STD or CTRL** in the corresponding reaction cups, add **200 µl of diluted TRACER** and mix well. Each treated standard or control is named **pre-incubate.**

9. TEST PROCEDURE

Carry out the test in duplicates.

1. Wash the microtiter plate **2 x with 250 µl** of wash buffer. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
2. Add **100 µl** of the pre-treated **STD** (standards), **CTRL** (controls) or **samples (corresponding pre-incubate)** into each well in duplicate.
3. Cover the strips and incubate for **2 hours** shaking on a horizontal shaker at room temperature (18-26°C).
4. Decant the contents of each well. Wash the microtiter plate **5 x with 250 µl** of wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution.
5. Add **100 µl** of **CONJ** (conjugate) into each well.
6. Cover the strips and incubate for **1 hour** shaking on a horizontal mixer at room temperature (18-26°C).

7. Decant the contents of each well. Wash the microtiter plate **5 x with 250 µl** of wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution.
8. Add **100 µl** of **SUB** (substrate solution) into each well.
9. Incubate for **10 - 20 minutes** at room temperature (18-26°C).
10. Add **100 µl** of **STOP** (stop solution) into each well and mix shortly in the ELISA reader using the shake option.
11. Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards **(STD)** is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used.

10. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend the use of the "4-Parameter-algorithm".

1. 4-parameter-algorithm

   It is recommended a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).
2. Point-to-point-calculation

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

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Serum and plasma samples

The estimated values are multiplied by a dilution factor of **10**.

11. LIMITATIONS

If the absorption of any sample is outside the measurement range, the sample should be diluted with SAMPLEBUF (sample dilution buffer) and re-assayed.

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

12. QUALITY CONTROL

Control samples should be analyzed 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.

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n=23)		
Sample	Myostatin [ng/ml]	Vk [%]
1	24.1	10.4
2	37.9	7.8

Inter-Assay (n=6)		
Sample	Myostatin [ng/ml]	Vk [%]
1	18.1	12.2
2	14.8	14.0

Linearity

Two patient serum samples were diluted and analyzed. The results are shown below:

n = 2

Sample	Dilution	Expected [ng/ml]	Measured [ng/ml]
A	1:10		30.4
	1:20	15.2	12.5
	1:40	7.6	8.7
B	1:10		29.0
	1:20	14.5	13.9
	1:40	7.3	8.2

Blank and calculated detection limit

The calculated detection limit (LoB; Limit of Blank) was set as B_0 + 1.645*SD. Standard 1 (blank) was measured 320 times.

LoB = 0.370 [ng/ml]

The detection limit was estimated based on the concentration from the calibration curve without considering the sample dilution factor.

14. PRECAUTIONS

- All kit reagents are for research use only.
- The quality control guidelines should be followed.
- Human material used in the kit components was 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.
- Stop solution is composed of sulphuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.

15. Technical Hints

- Do not mix different lot numbers of any kit component. Furthermore, do not assemble cavities of different microplates for analyses, even if the microplates are of the same charge.
- Reagents should not be used beyond the expiration date shown 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.
- The assay should always be performed according the enclosed manual.

16. General notes on the test and test procedure

- All reagents in the kit package are for research use only.
- 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 wrong use.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG together with a written complaint.

17. References

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Used symbols:


Temperature limitation

REF Catalogue Number

RUO For research use only


Contains sufficient for <n> tests


Manufacturer


Use by

LOT Lot number