Instructions for Use

Myostatin ELISA

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, use sólo la versión válida de las instrucciones de uso que se suministran con el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.
Por favor, usar a versão válida das instruções de utilização fornecidas com o kit.

Table of Contents

1 INTENDED USE

Myostatin ELISA is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of Myostatin in human serum and plasma.

The assay is for **research use only** and intended to be used by professional users in a laboratory environment. It can be performed manually. Not for use in diagnostic procedures.

2 INTRODUCTION

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

Myostatin was first identified in 1997 by McPherron et al. They found out that nullmutant 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).

Meanwhile, the correlation of circulating myostatin concentration with muscle mass and muscle function or physical performance has been shown in many studies. In addition, an influence of injury, inflammation, physical activity on myostatin concentration has been found (Baczek *et al* 2020).

Indications of possible research areas:

- Regulation of muscle growth & muscle degeneration
- Glucose absorption & acquired insulin resistance
- Cardiac energy homeostasis & ventricular hypertrophy
- Cancer
- Kidney function
- AIDS
- COPD

3 MATERIAL SUPPLIED

Label	Kit components	Quantity
PLATE	Microtiter plate, pre-coated	12 x 8 wells
WASHBUF	Wash buffer concentrate, 10x	2 x 100 mL
SAMPLEBUF	Sample dilution buffer, ready to use	1 x 100 mL
STD	Standards, lyophilised (see specification for concentrations)	2 x 5 vials
TRACER	Tracer concentrate, biotinylated myostatin	1 x 150 µL
CTRL 1	Control, lyophilised (see specification for range)	2 x 1 vial
CTRL 2	Control, lyophilised (see specification for range)	2 x 1 vial
CONJ	Conjugate concentrate, streptavidin-labelled peroxidase	1 x 200 µL
SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 mL
STOP	Stop solution, ready-to-use	1 x 15 mL

4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10 µL – 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)

* DRG 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 °C - 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 °C - 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 (75 µL TRACER + 7.5 mL SAMPLEBUF).
  The TRACER is stable at **2 °C - 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 °C - 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 °C - 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 °C - 8 °C**.

6 STORAGE AND PREPARATION OF SAMPLES

6.1 Sample storage

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

6.2 Preparation of samples

We recommend to carry out the tests in duplicates.

1. Pipet **15 µL** of each **serum or plasma** sample in the respective labelled 1.5 mL reaction tubes.
2. Add **135 µL** of **sample dilution buffer** to each sample, mix well. This results in a **dilution factor of 1:10**
3. Add **150 µL tracer** (diluted TRACER) to each **diluted sample**, mix well.
   The prepared sample is named **pre-incubate.**

6.3 Preparation of standards and controls

Transfer **150 µL standard or control** in the corresponding reaction tubes, add **150 µL tracer** (diluted TRACER), mix well. Each treated standard or control is also named **pre-incubate.**

7 ASSAY PROCEDURE

7.1 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 yellow 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 samples, is determined directly from this curve.

7.2 Test procedure

Bring all **reagents and samples to room temperature** (15 °C - 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 °C - 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 DRG.

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 °C - 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 °C - 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 °C - 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.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** 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

DRG 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 samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

10.1 Reference range

We recommend each laboratory to establish its own reference range.

11 PERFORMANCE CHARACTERISTICS

11.1 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

11.2 Dilution Recovery

Two serum samples were diluted and analyzed. 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.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.
- 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 harmful to health and the environment. Substrates for enzymatic color reactions can also cause skin and/or respiratory irritation. Any contact with the substances should be avoided. Further safety information can be found in the safety data sheet, which is available on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.
  Warning: Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.
- 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 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. DRG can therefore not be held responsible for any damage resulting from incorrect use.

15 REFERENCES

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SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Fertigungslosnummer, Charge *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Biological risks*	Biologische Risiken*	Rischi biologici	Riesgos biológicos	Risques biologiques
	Caution *	Achtung *	Attenzione	Precaución	Attention
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