





## Instructions for Use

# Titin Antibody ELISA

## Enzyme Immunoassay for the Determination of anti-Titin-Antibodies in Serum and Plasma

REF EA601/48

 6 x 8

 2 – 8 °C

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## 1. Introduction and Principle of the Test

In about 80 % of patients with Myasthenia gravis alterations of the thymus can be detected. Approximately 10 % of these patients develop thymus neoplasia as thymic epithelial tumor (TET) or thymic carcinoma. An early diagnosis of the thymoma and subsequent thymectomy is decisive for the prognosis of these patients.

Most of the affected patients develop acetylcholine receptor antibodies which can be detected with the ACHRAB<sup>®</sup>-Assay for the diagnosis of myasthenia gravis, as well as autoantibodies against striated muscles, among others antibodies to titin.

Titin is a protein of the striated muscles with an extremely high molecular weight. The immunogenic region of titin is located on a 30 kD protein fragment. Antibodies against this fragment presumably crossreact with the epitopes of the acetylcholine receptors (paraneoplastic myasthenia gravis). The recombinant MGT30 peptide is used in the ELISA for the specific determination of anti-titin antibodies.

The new ELISA assay is clearly superior to the immunofluorescence test on sections of striated human or monkey muscles.

The Anti-Titin ELISA kit uses the microtitre plate format. Recombinant titin fragment (MGT30 peptide) is coated onto the surface of the microwells. Diluted serum specimens are incubated to allow antibodies to titin to bind to the plastic surface. After washing away unbound antibodies and serum constituents, the specific titin antibodies are detected by protein A-peroxidase. The TMB / peroxidase reaction is monitored at 450 nm.

## 2. Precautions

- For in vitro research use only.
- Do not eat, drink or smoke where immunodiagnostic materials are being handled. Do not pipette by mouth.
- Some reagents contain sodium acid as preservative. Avoid skin contact.
- Wear disposable gloves when handling immunodiagnostic material.
- Some kit components are made with human sera. All sera used were tested for HIV I/II antibodies, HCV and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potential biohazardous material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.

## 3. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Allow all reagents to reach room temperature before use.

## 4. Contents of the Test

4.1	<b>MT-Strips</b> <b>STRIPS</b> 8 wells each, break apart precoated with recombinant MGT30 peptide	6 strips
4.2	<b>Enzyme Conjugate</b> <b>CONJ</b> 6 ml, ready for use Protein-A-POD conjugate	1 vial
4.3	<b>Calibrator</b> <b>CAL</b> 1 ml serum, ready for use (1:101 prediluted)	1 vial
4.4	<b>Negative Control</b> <b>CON -</b> 1 ml serum, ready for use (1:101 prediluted)	1 vial
4.5	<b>Positive Control</b> <b>CON +</b> 1 ml serum, ready for use (1:101 prediluted)	1 vial

4.6	<b>Sample Diluent</b> <b>DIL</b> 55 ml, ready for use	1 bottle
4.7	<b>Wash Buffer</b> <b>WASH</b> 50 ml, concentrated Dilute with dist. water to 500 ml.	1 vial
4.8	<b>Substrate</b> <b>SUB</b> 6 ml TMB solution, ready for use	1 vial
4.9	<b>Stop Solution</b> <b>STOPP</b> 6 ml, ready for use Contains 0.3M sulphuric acid, not corrosive	1 vial

**Reagents and materials required but not provided:**

- Pipettes (20 µl, 100 µl, 1 ml)
- Repeating dispenser 100 µl
- Horizontal shaker
- Microplate washing device
- Microplate photometer
- Distilled water

## 5. Preparation of Reagents and Samples

Allow all reagents and required number of MT strips to reach room temperature.

### 5.1 Specimen

Fresh plasma or serum samples are suitable. Samples should be stored at -20 °C if necessary. Repeated freezing and thawing, however, can affect the results. Hemolytic samples should not be used.

Samples must be diluted 1 : 101 (e.g. 10 µl serum plus 1 ml diluent) in Sample Diluent before assay. Diluted samples should be stored frozen for repeated measurement.

### 5.2 MT-Strips

**STRIPS**

Keep the closed bag at room temperature for around 10 minutes. Take the strips that are not needed out of the frame and store them in the thoroughly closed bag (leave the desiccant in the bag).

### 5.3 Wash Buffer

**WASH**

Prepare the Wash Buffer by diluting with distilled water to a final volume of 500 ml. Diluted Wash Buffer is stable for two months at 2 - 8 °C.

All other reagents are ready for use.

## **6. Assay Procedure**

### **6.1 Sample Incubation**

Dispense 100 µl of each ready for use Calibrator, ready for use Negative and Positive Control and diluted patient samples into the corresponding wells.

Incubate 60 minutes at room temperature on a horizontal shaker.

### **6.2 Washing**

Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl Wash Buffer. Repeat the washing procedure 2 to 3 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

### **6.3 Conjugate Incubation**

Dispense 100 µl Enzyme Conjugate into each well.

Incubate for 30 minutes at room temperature on a shaker.

### **6.4 Washing**

Repeat the washing procedure as described in 6.2.

### **6.5 Substrate Incubation**

Dispense each 100 µl Substrate in the wells and incubate for 15 to 25 minutes on a shaker.

### **6.6 Stopping**

Dispense each 100 µl Stop Solution into the wells in the same order sequence as the Substrate.

### **6.7 Measurement**

Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 10 minutes.

Samples with an OD higher than 2.5 at 450nm should be read again at 405nm and should be evaluated against the calibrator read at 405 nm.

## 7. Calculation and Interpretation of Results

The measured optical density of the samples divided by the optical density of the Calibrator equals factor F:

$$\text{Factor}_{\text{Sample}} = \frac{\text{O.D. Sample}}{\text{O.D. Calibrator}}$$

### Typical example:

	O.D. Sample	$\frac{\text{O.D. Sample}}{\text{O.D. Calibrator}}$	Interpretation
Calibrator	0.766	1.0	
Negative Control	0.126	0.2	-
Positive Control	2.157	2.8	+
Sample 1	0.391	0.5	-
Sample 2	1.498	2.0	+

Samples with an OD higher than 2.5 at 450nm should be read again at 405nm and should be related to the calibrator read at 405nm.

In the following table the results of the measurement of 100 sera of normal healthy blood donors are shown. The following picture shows the distribution of the normal values.

Furthermore the table shows first data of control groups (patients with ANA or TRAb antibodies, respectively) to check the specificity.

First measurements with sera from thymoma patients showed values from factor 2 up to 4.

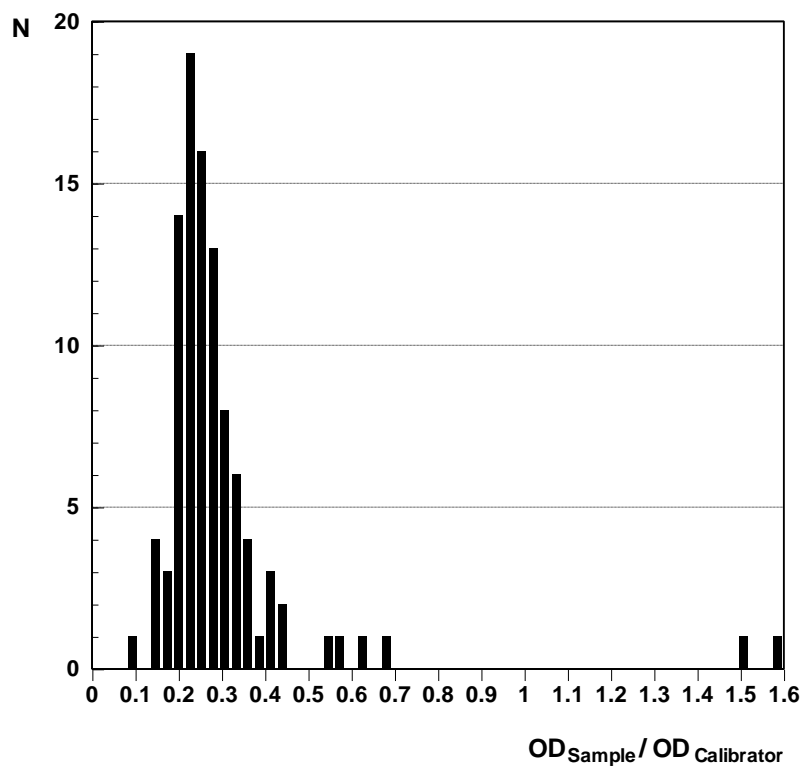
As a preliminary normal range owing to the obtained results we recommend concentrations < 1.0.



## Results

	Normal	ANA+	TRAb+
N	100	12	6
Min	0.09	0.27	0.20
Max	1.60	1.46	0.38
95%-Percentile	0.54	0.92	0.38
Median	0.25	0.45	0.26

### Distribution of normal values



## Reproducibility

Reproducibility was determined by measuring the intra- and inter-assay coefficients of variation.

Concentration is given as  $OD_{\text{sample}}/OD_{\text{calibrator}}$ .

### Intra Assay

sample	number n	mean	sd	cv (%)
1	40	2.30	0.053	2.3
2	40	0.52	0.015	2.8

### Inter Assay

sample	number n	mean	sd	cv (%)
1	11	0.10	0.012	12.0
2	11	0.16	0.024	15.0
3	11	0.19	0.030	15.8
4	11	0.99	0.082	8.3
5	11	1.40	0.114	8.1
6	11	1.79	0.186	10.4
7	11	2.51	0.207	8.2
8	11	3.39	0.401	11.8
9	11	3.56	0.336	9.4
10	11	4.63	0.632	13.7

## 8. Literature

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- RD Voltz, WC Albrich, A Nägele, F Schumm, M Wick, A Freiburg, M Gautel, HT Thaler, J Aarli, Th Kirchner, R Hohlfeld  
**Paraneoplastic myasthenia gravis: Detection of anti-MGT30 (titin) antibodies predicts thymic epithelial tumor**  
Neurology 1997;49:1454-1457
- M Gautel, A Lakey, DP Barlow, Z Holmes, S Scales, K Leonard, S Labeit, A Mygland, NE Gilhus, JA Aarli  
**Titin antibodies in myasthenia gravis: Identification of a major immunogenic region of titin**  
Neurology 1993;43:1581-1585

## Pipetting Scheme

Calibrator	100 $\mu$ l
Controls (negative + positive)	100 $\mu$ l
Samples 1:101 diluted	100 $\mu$ l



60 minutes incubation at room temperature



3 - 4 x washing



Enzyme Conjugate	100 $\mu$ l
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30 minutes incubation at room temperature



3 - 4 x washing



Substrate	100 $\mu$ l
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15 to 25 minutes incubation at room temperature



Stop Solution	100 $\mu$ l
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Reading of absorbance at 450 nm