



# anti-MAG Autoantibodies ELISA

## Procedure

EK-MAG

### Pre-Analytics

Specimen: Serum

Specimen storage: Serum samples are stable for  $\geq$  1 year if stored at  $\leq 20$  °C.

Freeze/thaw-cycles: Not recommended

### Critical Steps

#### Samples and sample preparation

Samples: Samples must not be hemolytic, lipemic or icteric

Sample preparation: Dilute samples - let set for 1 hour at 18-28 °C - put for 10 minutes on ice prior to pipetting.

#### ELISA

Reagents All reagents but TMB (substrate) must be used refrigerated.

Washing steps: Wash plate 4 x with refrigerated wash buffer prior to using it - empty wells completely.

Use „plate mode“ (serial dispensing followed by serial „aspiration“) for automated washing.

Incubations: Both, first and second incubation have to be performed at 2-8°C.

Incubation with TMB substrate has to be performed on a plate rotator at RT (18-28°C).

### Sample Preparation

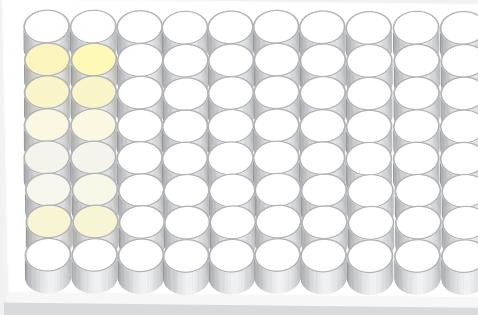
Dilute samples 1:1000 with incubation buffer.

Allow samples to set for 1 hour at 18-28 °C, vortex from time to time.

Put samples for 10 minutes on ice prior to pipetting.

### Microtiter Plate Set-up\*

BLANK (IB)  
CAL A  
CAL B  
CAL C  
CAL D  
Con L  
Con H



\*always perform ELISA measurements in duplicate

### ELISA

All reagents but TMB (substrate) must be used refrigerated

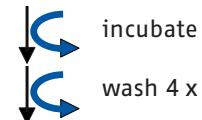
#### Precoated Microtiter Plate



wash 4 x

#### add 100 µl of Controls and diluted Samples (1:1000)

2 h

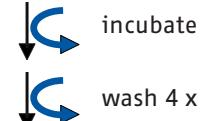


incubate 2 hrs ( $\pm 5$  min) at 2-8°C

wash 4 x

#### add 100 µl of Enzyme Label

2 h



incubate 2 hrs ( $\pm 5$  min) at 2-8°C

wash 4 x

#### add 100 µl of TMB Substrate

30'



incubate 30 ( $\pm 5$  min) at 18-28°C on a plate rotator

#### add 100 µl of Stop Solution

4.5 h → read at 450 nm (within 30 min)

Time to Result: ~5 h



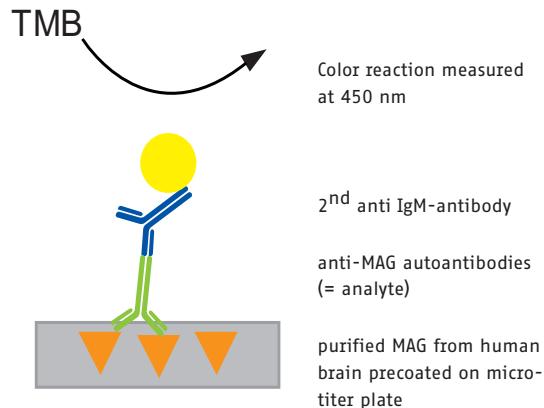
# anti-MAG Autoantibodies ELISA

Characteristics EK-MAG

## Intended Use

The anti-MAG Autoantibodies ELISA is intended for the quantitative *in vitro* diagnostic determination of human IgM autoantibodies directed against Myelin Associated Glycoprotein (=MAG).

## Assay Principle



## Kit Formats

Code	Enzyme label	Wells
EK-MAG	IgM	1 x 96

## Number of Tests in different Runs

Number of Runs	Number of Tests
1	41
2	34
3	27
4	20

## Assay Performance Data

### Intra-assay precision

6.5 %

The intra-assay precision was calculated from the results of n = 20 pairs of values obtained in a single run (CV range: 2.1 - 10.3%).

### Inter-assay precision

15.4 %

The inter-assay precision was calculated from the results of n = 20 results of pairs of values obtained in 20 different runs.

### Dilution Linearity / Parallelism

147%

Human serum samples with high titers of anti-MAG autoantibodies were diluted from 1:1000 to 1:64000. It is suggested that the relatively high deviation in about 50% of samples is due to antibody aggregation.

### Cut-off value

1000 BTU

The cut-off value for anti-MAG autoantibodies was determined from samples from n = 150 asymptomatic volunteer blood donors from 18-70 years of age. The mean +3SD values resulted in a technical cut-off value of 729 BTU. For practical reasons we recommend to use a cut-off value of 1000 BTU.

## Example of Results

EK-MAG (IgM)	absorbance (mean OD450)	BTU	% CV
Blank	0.048		
CAL A	2.191	70000	0.2
CAL B	1.258	15000	1.5
CAL C	0.408	3000	2.9
CAL D	0.134	1000	1.5
CRTL LOW	0.368	2666	3.1
CRTL HIGH	1.389	18261	0.6

## Results

The absorbance measured is proportional to the titer of anti-MAG autoantibodies present in a given sample. The titers of the quantitative anti-MAG Autoantibodies ELISA are expressed as BÜHLMANN Titer Units (BTU).

## Standardization

The calibrators of the BÜHLMANN anti-MAG Autoantibodies ELISA kit were calibrated against an internal reference consisting of more than 10 human sera with low to high titers of anti-MAG antibodies.

Ordering code:  
EK-MAG 96 tests (IgM)

CE-marked product



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