anti-MAG Autoantibodies ELISA

Procedure

**Pre-Analytics**

- **Specimen:** Serum
- **Specimen storage:** Serum samples are stable for ≥ 1 year if stored at ≤ 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**

- **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**

1. **Precoated Microtiter Plate**
   - Add 100 µl of Controls and diluted Samples (1:1000)

2. **Time to Result:** ~5 h

   - **2 h**
     - Incubate 2 hrs (±5 min) at 2-8°C
     - Wash 4 x
     - Add 100 µl of Enzyme Label
     - Wash 4 x
     - Add 100 µl of TMB Substrate
     - Incubate 30 (±5 min) at 18-28°C
     - On a plate rotator

3. **Read at 450 nm (within 30 min)**

4. **Time to Result:** ~5 h

*always perform ELISA measurements in duplicate
anti-MAG Autoantibodies ELISA

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

TMB

Color reaction measured at 450 nm

2nd anti IgM-antibody

anti-MAG autoantibodies (= analyte)

purified MAG from human brain precoated on microtiter plate

Kit Formats

<table>
<thead>
<tr>
<th>Code</th>
<th>Enzyme label</th>
<th>Wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>EK-MAG</td>
<td>IgM</td>
<td>1 x 96</td>
</tr>
</tbody>
</table>

Number of Tests in different Runs

<table>
<thead>
<tr>
<th>Number of Runs</th>
<th>Number of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>absorbance (mean OD450)</th>
<th>BTU</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAL A</td>
<td>2.191</td>
<td>70000</td>
<td>0.2</td>
</tr>
<tr>
<td>CAL B</td>
<td>1.258</td>
<td>15000</td>
<td>1.5</td>
</tr>
<tr>
<td>CAL C</td>
<td>0.408</td>
<td>3000</td>
<td>2.9</td>
</tr>
<tr>
<td>CAL D</td>
<td>0.134</td>
<td>1000</td>
<td>1.5</td>
</tr>
<tr>
<td>CRTL LOW</td>
<td>0.368</td>
<td>2666</td>
<td>3.1</td>
</tr>
<tr>
<td>CRTL HIGH</td>
<td>1.389</td>
<td>18261</td>
<td>0.6</td>
</tr>
</tbody>
</table>

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).

Standarization

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 (IgM) 96 tests (analyte)