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Product-No: MAG001 (8 determinations)
MAG002 (16 determinations)
MAG003 (24 determinations)

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ravo MAG-Blot
Immunoblot for the detection of anti-MAG IgM-autoantibodies

8, 16 or 24 determinations

Summary:

The myelin-associated glycoprotein (MAG) 1-5 is a transmembrane protein with a molecular weight of 100 kDA which exists in two isoforms (L-MAG-und S-MAG).
MAG represents approximately 0.1 – 1 % of the total myelin proteins of the peripheral and central nervous system.

Anti-MAG autoantibodies are detected in 50 % of patients with peripheral demyelinating neuropathies associated with a monoclonal IgM-gammopathy (Waldenström's macro-globulinemia and MGUS = monoclonal gammopathie of undetermined significance). The detection of anti-MAG autoantibodies in these diseases is of diagnostic relevance.

The autoantibodies are directed against epitopes of the carbohydrate part of MAG.
One of these epitopes (HNK1) is also expressed on the glycolipids SGPG and SGLPG.

There seems to be a correlation between the anti-MAG autoantibody titer and the grade of demyelinisation.

Anti-MAG autoantibodies, without diagnostic meaning, are rarely found in patients with multiple sclerosis, Guillain-Barré-Syndrom, chronic neuropathies and Myasthenia gravis.

Principle:

Nitrocellulose strips which are coated with highly purified MAG (from bovine brain) are incubated with a specimen of preabsorbed patient serum. Specific antibodies in the specimen will bind to the antigens. Non specific molecules in serum specimens will be removed by washing the strips. Bound antibodies are detected by alkaline phosphate conjugated anti-human IgM using BCIP/NBT as substrate.

Reagents:

- Nitrocellulose-Strips coated with highly purified MAG (from bovine brain)
- 1 control-scan
- IgG-Absorbent (RF-Stripper), ready to use, white screw cap
- Positive control, ready to use, contains 0.01% ProClin300, red screw cap
- Wash buffer concentrate, blue screw cap
- Dilution Buffer for samples, ready to use, contains 0.01% ProClin300, green screw cap
- Alkaline Phosphatase conjugated anti-human-IgM, ready to use, contains 0.01% ProClin300, yellow screw cap
- Substrate Solution (BCIP/NBT), ready to use, black screw cap
- Incubation tub(s)
- Instructions for use

Specimen and storage:

Serum is the recommended sample. Lipemic, hemolytic and icteric samples should not be used.
Serum samples can be stored at least for one week at + 4....8°C. For long term storage aliquots should be stored at < - 20°C. Avoid repeated freeze-thaw cycles.
All kit components (unopened and opened) are stable until date of expiry stored at + 4....8°C.

Reconstitution:

- Make sure all kit components are at room temperature before use. Dilute the wash buffer concentrate 1:20 with distilled water. **During storage at low temperatures, crystals may form in concentrated wash buffers, which can be dissolved by incubating the concentrate at 37°C for 30 minutes.** Diluted wash buffer is stable for 4 weeks stored at +4 to +8°C.

Absorption of serum samples with IgG-Absorbent (RF-Stripper)

- Pipet 80 µl IgG-Absorbent into a tube (0.5 ml Eppendorf cup).
- Add 20 µl of patient serum to the IgG-Absorbent.
- Thoroughly mix the sample by vortexing.
- Let the tube stand at room temprature for 30 minutes.

- Mix the sample again by gentle vortexing.
- Centrifuge at 10,000 rpm for 3 minutes at room temperature.
- Use immediately 20 µl of the clear supernatant for the test. Do not use the pellet!

Procedure:

- The nitrocellulose strips are labeled at the bottom (if not indicated otherwise). They must be incubated with the labels facing upwards and should be completely covered with fluid during all incubation steps.

Add 2 ml ready to use positive control to one strip.
Cover the other strips with 2 ml dilution buffer. Add 20 µl of absorbed serum sample and mix carefully.
Incubate for 60 minutes at room temperature on a rocking table.

- Wash three times for three minutes with diluted wash buffer.
- Add 2 ml alkaline phosphatase IgM conjugate, ready to use, per strip.

Incubate for 60 minutes at room temperature on a rocking table.

- Wash three times for three minutes with diluted wash buffer.
- Incubate each strip in 2 ml ready to use substrate-solution.

Incubate for 10 minutes at room temperature until the bands become clearly visible. See control scan for comparison.

Attention: prolonged incubation times may lead to nonspecific binding.

- Transfer the strips to distilled water to stop the reaction and wash the strips once with distilled water. Put the strips onto filter paper and let them dry. Store the strips in the dark.

Interpretation:

Put the strips side by side to assign the proteins. Only strips from the same batch should be compared.
The positive control and the control scan help to assign the proteins and to stick the strips on the latter for documentation.

Positive result:	Intensity of the band > cut-off control line
Borderline result:	Intensity of the band = cut-off control line
Negative result:	Intensity of the band < cut-off control line

There seems to be a coorelation between the anti-MAG autoantibody titer and the grade of demyelinisation.

Remarks:

- Avoid contact of the skin with substrate solution.