Anti-Myelin Auto-Antibodies ELISA

Enzyme Immunoassay for the Quantitative Measurement of

Human Antibodies against Myelin Antigens

English

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Symbols

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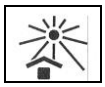		Keep away from sunlight / Nicht dem Sonnenlicht aussetzen
		Incubation time / Inkubationszeit
°C		Incubate at / Inkubation bei
↔		Shaking / schütteln
MTP		Miktrotiterplate/Mikrotiterplatte
Rec in		Reconstitute in / Rekonstituieren in
SPE		Sample / Probe
AbIgG	AK IgG	Antibody-Biotin-Conjugate AK IgG / Antikörper-Biotin-Konjugat AK IgG
AbIgM	AK IgM	Antibody-Biotin-Conjugate AK IgM / Antikörper-Biotin-Konjugat AK IgM
Conj	EK	Streptavidin-HRP-Conjugate EK / Streptavidin-POD-Enzymkonjugat EK
DILU X	VP	Dilute in Buffer X / Verdünnen in Puffer X
CAL X	STD 1/ STD 2	Standard X / Standard X
WASHBUF 20x	WP	Washing Buffer Concentrate / Waschpufferkonzentrat
WASHBUF		Washing Buffer / Waschpuffer
SUBST TMB	S	Substrate
H_2SO_4	SL	Stop Solution / Stopp Lösung
TAPE		Cover Plate with sealing tape / Platte abkleben
MEASURE		Measure plate within 30 min at 450 nm (Referencefilter ≥590nm) / Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm).

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PACKAGE INSERT

INTRODUCTION

Several neurodegenerative diseases are at least partially caused by auto immunreactions. Inflammatory processes result in neuronal loss. Mostly, T-cell dependent immunity is in the central part of the pathological concept. But it is known that in some neurological diseases like multiple sclerosis B-cell dependent immunity is also of relevance in disease progress.

Thus, antibodies are a well established means in differential diagnostics of neuronal diseases e.g. by the detection of oligoclonal bands or measurement of specific antibodies against neuronal Proteins like aquaporin IV.

This assay allows the quantitative detection of antigen specific IgG and IgM antibodies in serum samples and cerebrospinalfluid.

TESTPRINCIPLE

Myelin-specific proteins have been purified from bovine brain and used for coating of microtiterplates:

Myelin-oligodendrocyticglycoprotein (MOG); Myelin Basic Protein (MBP); Myelin-associated Glycoprotein (MAG); Proteolipoprotein (PLP); Alpha-B-Crystallin (CRY)

The antibodies in the samples bind specifically to the immobilized antigens. IgG- or IgM specific biotinylated antibodies together with a streptavidin peroxidase conjugate are employed for the detection of the bound antibodies of the sample. A chemical reaction catalyzed by the peroxidase results in a colour change of the substrate, which is stopped after defined period of time. The colour intensity correlates positive with the amount of specific antibodies bound.

To assess the quantity in each run a ready-to-use diluted human serum is used for calibration. The amount of specific antibody in the standard has been determined by titration. Thus, this assay quantifies the specific antibodies in the sample in arbitrary Mediagnost Units.

Specimen: Storage, Preparation, Transport

Non hemolytic, non icteric and non lipemic serum and cerebrospinal fluid samples can be used in this assay.

Specimen transport within 3 days can be done at room temperature: 20-25°C

Generally, specimens should be cooled as soon as possible. For longterm storage the samples should stored at -20°C or below. Repeated freeze/thawing cycles have to be avoided.

Usually a dilution of 1:1000-2000 in dilution buffer VP is sufficient for serum samples. In case of cerebrospinal fluid a dilution of 1:2.5 –1:10 is recommended.

MATERIAL

1)	MTP BSA,MOG, MBP, MAG, PLP,CRY	**2 Mikrotiterplates, each with 2 stripes** coated with BSA-Control (BSA), marked light blue Myelin-oligodendrocyticglycoprotein (MOG), marked black Myelin Basic Protein (MBP), marked lilac Myelin-associated Glycoprotein (MAG), marked orange Proteolipoprotein (PLP), marked green Alpha-B-Crystallin (CRY), marked dark blue
2)	DILU	**Dilution Buffer VP**, 2× 50 ml, ready-to-use: dilution of the
3)	CAL 1-2	**Standard 1 and 2 (STD1, STD2),** 100µL, 100fold concentrate, contain human serum.
4)	Ab IgG	**Antibody-Biotin-Conjugate AK IgG**, 12 ml, **ready-to-use**, contains the biotinylated anti-human IgG antibody. Please use 100 µl for each well.
5)	Ab IgM	**Antibody-Biotin-Conjugate AK IgM**, 12 ml, **ready-to-use**, contains biotinylated anti-human IgM Antibody. Please use 100 µl for each well.
6)	Conj	**Streptavidin-HRP-Conjugate EK**, 2× 12 ml, **ready-to-use**, contains the streptavidin peroxidase conjugate. Please use 100 µl for each well.
7)	WASHBUF 20x	**Washing Buffer WP,** 2×50 ml, 20-fold concentrate, please dilute before use **1:20 with A.dest** or demineralized water (e.g. put the content of one bottle of 50 ml in a graduated cylinder and fill up with water to 1000 ml). Please dilute Washing Buffer WP according to your requirements, use max. 4 weeks.
8)	SUBST	**Substrate (S),** 2× 12 ml, **ready for use**, horseradish-peroxidase-(HRP)-substrate. Use 100 µl pro well in the assay
9)	H_2SO_4	**Stopping Solution (SL),** 2× 12 ml, **ready for use**, 0.2 M sulphuric acid, Caution acid! Use 100 µl pro well in the assay
10)		Sealing tape for covering of the microtiter plate, 6 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes (100 and 200µl) Micropipettes and multichannel pipettes with disposable plastic tips
Distilled or Deionized water for dilution of the Washing Buffer (WP)
Vortex-mixer
Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human samples)
Timer (120 min. range)
Reservoirs (disposable)
Plate washer and plate shaker (recommended)
Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620nm (or ≥590 nm)
Foil welding device for laminate bags (recommended)

WARNINGS AND PRECAUTIONS

For research and professional use only.
The Mediagnost kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Mediagnost will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.

Do not use obvious damaged or microbial contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.

Human Serum
Following components contain human serum: **STD 1** and **STD 2**
Source human serum for the control sera provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient´s specimens should be treated as potentially infectious.

Reagents AK-IgG, AK-IgM, EK, VP, WP
Contain as preservatives f **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

Substrate Solution (S)
The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbencidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

Stopping Solution (SL)
The Stopping solution contains 0.2 M acid sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.
Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.
Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

Technical Recommendations

The assay has to be conducted strictly according the test protocol herein.

The reagents are stable until the indicated expiry, if stored unopened and protected from sunlight at 2 – 8°C.

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and/ or warming.

Incubation at room temperature means: 20-25°C

Standards

Standards are provided as 100fold concentrate and have to be diluted 1:100 in VP before use.

Washing Buffer

The required volume of washing buffer is prepared by 1:20 dilution of the provided 20-fold concentrate with deionised water. The diluted Washing Buffer is stable for max. 4 weeks at 2-8°C.

Microtiterplates

Unused microtiterplate wells have to be stored airtight together with the desiccant bag at 2-8°C and used in the frame provided. The labelled expiry is not influenced in case of proper storage.

Substrate

The **Substrate Solution S**, is photosensitive – store and incubate in the dark.

ASSAY PROCEDURE

NOTES: For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay, the standards and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, **Antibody Conjugate (AK IgG) or (AK IgM), Enzyme Conjugate EK, Substrate Solution S** as well as the **Stop Solution SL** should be added to the plate in the same order and in the same time interval each, respectively. Before beginning the test procedure bring all reagents to room temperature.

According to your requirements you can choose which antibodies are measured. The kit contains two microtitre plates, each contains two stripes coated with each antigen. The stripes are colour coded and the code is shown below. The Specificity-Control (BSA-coated stripes) allows to measure the sample dependent unspecific binding.

Coating:	**Colour:**
Column 1 BSA Control	light blue
Column 2 MOG	black
Column 3 MBP	lilac
Column 4 MAG	orange
Column 5 PLP	green
Column 6 CRY	dark blue

Columns 7 – 12 are filled in the same sequence as above. We describe the test procedure for this setting below.

1) Pipette in positions A1-A6 each **100 µl Dilution Buffer VP** (Blank), pipette in positions B1-B6 each **100 µl prediluted Standard 1** and pipette in positions C1-C6 each **100 µl predilutedStandard 2**.

Pipette **100 µl** of the **diluted samples** (generally 1:1000 diluted in Dilution Buffer **VP**) in the rest of the wells according to your requirements.
For example: Sample 1: in positions D1-D6, Sample 2: in E1-E6, Sample 3: in F1-F6, Sample 4: in G1-G6, Sample 5: in H1-H6, Sample 6: in A7-A12 etc.

Examplary pipetting schedule:

	1 BSA	2 MOG	3 MBP	4 MAG	5 PLP	6 CRY	7 BSA	8 MOG	9 MBP	10 MAG	11 PLP	12 CRY
A	VP	VP	VP	VP	VP	VP	Sample 6	Sample 6	Sample 6	Sample 6	Sample 6	Sample 6
B	STD1	STD1	STD1	STD1	STD1	STD1	Sample 7	Sample 7	Sample 7	Sample 7	Sample 7	Sample 7
C	STD2	STD2	STD2	STD2	STD2	STD2	Sample 8	Sample 8	Sample 8	Sample 8	Sample 8	Sample 8
D	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 9	Sample 9	Sample 9	Sample 9	Sample 9	Sample 9
E	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 10	Sample 10	Sample 10	Sample 10	Sample 10	Sample 10
F	Sample 3	Sample 3	Sample 3	Sample 3	Sample 3	Sample 3	Sample 11	Sample 11	Sample 11	Sample 11	Sample 11	Sample 11
G	Sample 4	Sample 4	Sample 4	Sample 4	Sample 4	Sample 4	Sample 12	Sample 12	Sample 12	Sample 12	Sample 12	Sample 12
H	Sample 5	Sample 5	Sample 5	Sample 5	Sample 5	Sample 5	Sample 13	Sample 13	Sample 13	Sample 13	Sample 13	Sample 13

2) Cover the wells with the sealing tape and incubate the plate for **2 hours** at **room temperature.**

3) After incubation aspirate the contents of the wells and wash the wells **3 times** with **300 µl Washing Buffer WP**.

1) Following the last washing step, pipette **100 µl Antibody Conjugate AK (AK-IgG or AK-IgM)** dependent on your requirements (Measurement of IgG or IgM antibodies!) in each well.

2) Cover the wells with the sealing tape and incubate **1 hour** at **room temperature.**

3) After incubation wash the wells 3 times with **Washing Buffer WP** (see step 3).

4) Following the last washing step, pipette **100 µl Enzyme Conjugate EK** in each well.

5) Cover the wells with the sealing tape and incubate **1 hour** at **room temperature.**

6) After incubation wash the wells 3 times with **Washing Buffer WP** (see step 3).

7) Pipette **100 µl of the TMB-Substrate solution S** in each well.

8) Incubate the plate for **15 minutes** in the dark at room temperature

9) After incubation pipette **100 µl Stop Solution SL** in each well.

10) Measure the absorbance **within 30 minutes at 405 nm (Reference filter ≥590 nm, e.g. 620 nm)**.

CALCULATION OF RESULTS

Calibration Curve

For the evaluation of the assay it is required that the absorbance values of the blank (antigen specific blanks A1-6) should be below 0.50.

The extinction of the blanks and the extinction of the nonspecific binding (Column 1 and Column 7) have to be substracted from the sample extinctions (Column 2-6 and Column 8-12), respectively.

For calibration 100fold standard concentrates (STD1 and STD 2) are enclosed in the kit. The quantity of specific antibodies of the finally diluted standards has been determined by titration and the results are shown in Table 1.

Table 1: Standard concentration of IgG and IgM [Mediagnost Unit/mL]

	IgG Titer		IgM Titer	
	STD1	**STD2**	**STD1**	**STD2**
MOG	2.05	8.21	4.10	16.4
MBP	3.12	12.46	2.95	11.8
MAG	0.37	1.46	0.53	2.12
PLP	1.71	6.82	4.90	19.6
CRY	0.66	2.65	9.11	10.6

Use the origin and the two standards to plot a calibration curve.
Recommendation: Calculation of the standard curve should be done by using a computer program. The curve is in general described well by linear regression (y = mx).

The concentration in Mediagnost Units/mL of the samples can be calculated by multiplication with the respective dilution factor.

Because of the proven linearity (1:1000 -1:100000) of sample dilution, the calibration curve can be extrapolated to calculate the antibody content of samples with a singal above STD2. But we recommend to measure these samples again in a higher dilution.

Test Characteristics

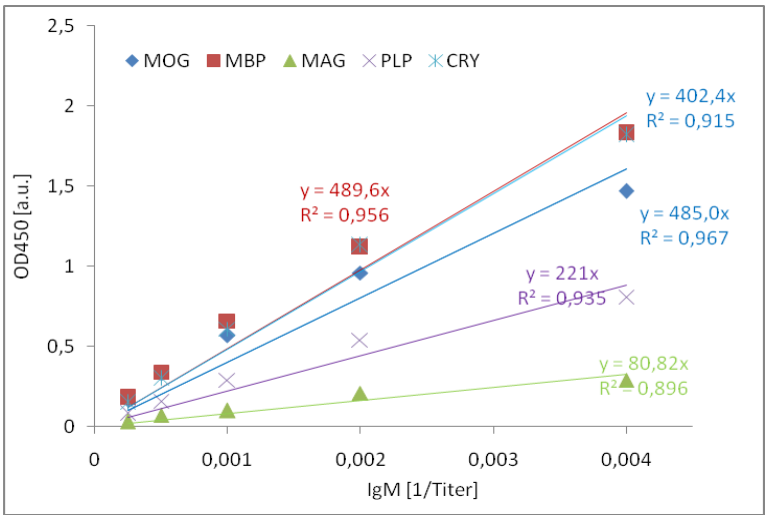
Reproducibility and Precision
Inter-assay variability was tested by doing five measurements of three different serum samples and for intra-assay variance, serum samples were measured 6 times in the same assay.

	MOG	**MBP**	**MAG**	**PLP**	**CRY**
Inter-Assay IgG Mean [%CV]	13.77	7.17	10.75	17.47	9.30
SD Inter-Assay IgG SD	6.61	1.94	6.70	3.84	4.17
Inter-Assay IgM Mean [%CV]	14.84	12.74	14.07	15.64	14.14
SD Inter-Assay IgM SD	2.87	6.36	3.11	4.19	2.85
Intra-Assay IgG Mean [%CV]	3.94	3.76	7.43	8.42	3.73
SD Intra-Assay IgG SD	1.83	1.49	3.20	5.82	0.66
Intra-Assay IgM Mean [%CV]	4.66	3.95	11.35	6.31	5.43
Intra-Assay IgM SD	2.59	2.58	2.93	2.92	3.81

Linearity of Dilution

Linearity of sample dilution of a representative sample. Here the arbitrary IgM (a) and IgG (b) optical densities are shown. The measured optical density [arbitrary units; a.u.] was laid on against the dilution [1/Titer]. Data were analysed by linear regression. Resulting equations and correlation factors are shown.

a)



b)