

Instructions for Use

Aquaporin-4 Autoantibody ELISA

IVD



REF EIA-5431

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***Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.***

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1 INTENDED USE

The Aquaporin-4 Autoantibody ELISA kit is intended for use by professional persons only, for the quantitative determination of AQP4 autoantibodies (AQP4 Ab) in human serum.

Neuromyelitis optica (NMO), also known as Devic's syndrome, is an immune-mediated neurologic disease that involves the spinal cord and optic nerves. It can be considered to be a disorder distinct from multiple sclerosis (MS). A serum immunoglobulin G autoantibody (NMO-IgG) has been shown to be a specific marker for NMO and the water channel aquaporin 4 (AQP4) has been identified as the antigen for NMO IgG. Measurement of AQP4 Ab can be of considerable value in distinguishing NMO from MS when full clinical features may not be apparent and early intervention may prevent or delay disability.

2 REFERENCES / LITERATURE

1. V. A. Lennon et al.
A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis.
Lancet 2004 364(9451): 2106 - 2112
2. V. A. Lennon et al.
IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel.
The Journal of Experimental Medicine 2005 202: 473 - 477
3. B. G. Weinschenker et al.
Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis.
Annals of Neurology 2006 59: 566 - 569
4. N. Isobe et al.
Quantitative assays for anti-aquaporin-4 antibody with subclass analysis in neuromyelitis optica.
Multiple Sclerosis Journal 2012 18: 1541 – 1551
5. S. Jarius et al.
Testing for antibodies to human aquaporin-4 by ELISA: Sensitivity, specificity and direct comparison with immunohistochemistry.
Journal of the Neurological Sciences 2012 320:32 - 37

PATENTS

The following patents apply:

European patent EP 1 700 120 B1, US patents US 7,101,679 B2, US 7,947,254 B2 and US 8,889,102 B2, Chinese patent ZL200480040851.3 and Japanese patent 4538464.

3 ASSAY PRINCIPLE

In Aquaporin-4 Autoantibody ELISA kit, AQP4 Ab in patients' sera, calibrators and controls are allowed to interact with AQP4 coated onto ELISA plate wells and liquid phase biotinylated AQP4 (AQP4-Biotin). After incubation at room temperature for 2 hours with shaking, the well contents are discarded. AQP4 Ab bound to the AQP4 coated on the well will also interact with AQP4-Biotin through the ability of AQP4 Ab in the samples to act divalently leaving AQP4-Biotin bound to the well via an AQP4 Ab bridge. The amount of AQP4-Biotin bound is then determined in a second incubation step involving addition of streptavidin peroxidase (SA-POD), which binds specifically to biotin. Excess, unbound streptavidin peroxidase is then washed away and addition of the peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (TMB), results in formation of a blue colour. This reaction is stopped by the addition of a stop solution, causing the well contents to turn yellow. The absorbance of the yellow reaction mixture at 450 nm and 405 nm is then read using an ELISA plate reader. A higher absorbance indicates the presence of AQP4 autoantibody in the test sample. Reading at 405 nm allows quantitation of high absorbances.

It is recommended that values below 10 U/mL should be measured at 450 nm. If it is possible to read at only one wavelength 405 nm may be used.

The measuring interval is 3.0 – 80 U/mL (arbitrary DRG units).

4 STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below -20 °C. 100 µL is sufficient for one assay (duplicate 50 µL determinations).

Repeated freeze thawing or increases in storage temperature should be avoided. Do not use lipaemic or haemolysed samples.

Studies in which EDTA, citrate and heparin plasma samples were spiked with AQP4 Ab positive sera showed minor changes in signal compared with spiked serum from the same donor. In particular OD₄₅₀ values with spiked EDTA, citrate and heparin plasmas were 79% - 128% of spiked serum (15 samples with serum concentrations ranging from 2.6 U/mL – 30 U/mL) or 87% – 130% in terms of U/mL.

When required, thaw test sera at room temperature and mix gently to ensure homogeneity.

Centrifuge serum prior to assay (preferably for 5 min at 10-15,000 rpm in a microfuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

5 MATERIALS REQUIRED AND NOT SUPPLIED

- Pipettes capable of dispensing 25 µL, 50 µL and 100 µL.
- Means of measuring various volumes to reconstitute or dilute reagents supplied.
- Pure water.
- ELISA Plate reader suitable for 96 well formats and capable of measuring at 450 nm and 405 nm.
- ELISA Plate shaker, capable of 500 shakes/min (not an orbital shaker).
- ELISA Plate cover.

6 PREPARATION OF REAGENTS SUPPLIED

Store unopened kit and all kit components at 2 °C - 8 °C.

A	AQP4 Coated Wells 12 breakapart strips of 8 wells (96 in total) in a frame and sealed in foil bag. Allow foil bag to stand at room temperature (20 °C - 25 °C) for 30 minutes before opening.
	Ensure wells are firmly fitted in the frame provided. After opening return any unused wells to the original foil bag and seal with adhesive tape. Then place foil bag in the self-seal plastic bag with desiccant provided and store at 2 °C - 8 °C for up to kit expiry date.
B1-5	Calibrators 1.5, 5, 20, 40, 80 U/mL (arbitrary DRG units) 5 x 0.7 mL Ready for use
C1-2	Positive Controls I & II (see label for concentration range) 2 x 0.7 mL Ready for use
D	Negative Control 0.7 mL Ready for use
E	AQP4–Biotin 3 vials Lyophilised
	Immediately before use, reconstitute with reconstitution buffer for AQP4-Biotin (F), 1.5 mL per vial. When more than one vial is to be used, pool the contents of the vials and mix gently.
F	Reconstitution Buffer for AQP4-Biotin 10 mL Ready for use
G	Streptavidin Peroxidase (SA-POD) 0.8 mL Concentrated
	Dilute 1 in 20 with diluent for diluting SA-POD (H). For example, 0.5 mL (G) + 9.5 mL (H). Store for up to 16 weeks at 2 °C - 8 °C after dilution.
H	Diluent for SA-POD 15 mL Ready for use
I	Peroxidase Substrate (TMB) 15 mL Ready for use
J	Concentrated Wash Solution 120 mL Concentrated
	Dilute 1 in 10 with pure water before use. Store at 2 °C - 8 °C up to kit expiry date.
K	Stop Solution 14 mL Ready for use

7 ASSAY PROCEDURE

Allow all reagents to stand at room temperature (20 °C - 25 °C) for at least 30 minutes prior to use.

Do not reconstitute AQP4-Biotin until step 2 below.

An Eppendorf type repeating pipette is recommended for steps 2, 5, 8, and 9.

1. Pipette **50 µL** (in duplicate is recommended) of patient sera, calibrators (B1-5) and controls (C1-2 and D) into respective wells. Leave one well empty for blank.
2. Reconstitute AQP4-Biotin and pipette **25 µL** into each well (except blank).
3. Cover the frame and shake the wells for 2 hours at room temperature on an ELISA plate shaker (500 shakes per min).
4. Use an ELISA plate washer to aspirate and wash the wells three times with diluted wash solution (J). If a plate washer is not available, discard the well contents by briskly inverting the frame of wells over a suitable receptacle, wash three times manually and tap the inverted wells gently on a clean dry absorbent surface to remove excess wash.
5. Pipette **100 µL** of diluted SA-POD (G) into each well (except blank).
6. Cover the plate and incubate for 20 minutes at room temperature on an ELISA plate shaker (500 shakes per min).
7. Repeat wash step 4. If manual washing is being carried out, use pure water for the final wash step (to remove any foam) before tapping the wells dry.
8. Pipette **100 µL** of TMB (I) into each well (including blank) and incubate for 20 minutes in the dark at room temperature without shaking.
9. Pipette **100 µL** stop solution (K) into each well (including blank) and shake the plate for approximately 5 seconds on a plate shaker. Ensure substrate incubations are the same for each well.
10. Within 10 minutes, read the absorbance of each well at 450 nm and 405 nm using an ELISA plate reader, blanked against a well containing **100 µL** of TMB (I) and **100 µL** stop solution (K) **only**.

8 RESULT ANALYSIS

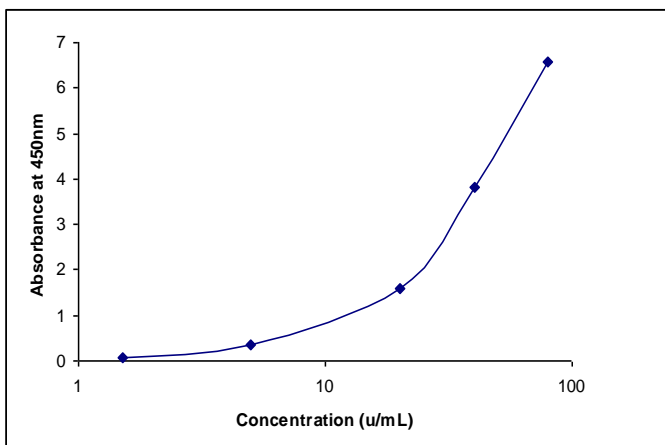
A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the absorbance of the calibrators on the y-axis (linear scale). The AQP4 Ab concentrations in patient sera can then be read off the calibration curve [plotted at DRG as a spline log/lin curve (smoothing factor = 0)]. Other data reduction systems can be used.

The negative control can be assigned a value of 0.15 U/mL to assist in computer processing of assay results.

Samples with AQP4 Ab concentrations above 80 U/mL can be diluted (e.g. 10 x and/or 100 x) in AQP4 Ab negative serum. Some sera will not dilute in a linear way.

TYPICAL RESULTS (Example only; not for calculation of actual results)

	A 450 nm	Conc. U/mL	A 405 nm	Conc. U/mL
Negative Control (D)	0.021		0.006	
B1	0.085	1.5	0.024	1.5
B2	0.354	5	0.108	5
B3	1.577	20	0.468	20
B4	3.802	40	1.118	40
B5	6.588	80	1.938	80
Positive Control (CI)	0.755	12.1	0.224	12.0
Positive Control (CII)	2.506	28	0.745	28



9 ASSAY CUT OFF

Negative	< 3.0 U/mL
Positive	≥ 3.0 U/mL

This cut off has been validated at DRG. However each laboratory should establish its own normal and pathological reference ranges for AQP4 Ab levels. Also it is recommended that each laboratory include its own panel of control samples in the assay.

10 CLINICAL EVALUATION

(The information below is derived from 450 nm data)

10.1 Clinical Specificity

Sera from 358 individual healthy blood donors were tested in the Aquaporin-4 Autoantibody ELISA kit. 356 (99%) sera were identified as being negative for AQP4 Ab.

10.2 Clinical Sensitivity

Of 62 sera from patients with NMO or NMO spectrum disorder (NMOSD) 48 (77%) were positive for AQP4 Ab.

10.3 Lower Detection Limit

The negative control was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at 2 standard deviations was 0.17 U/mL.

10.4 Intra Assay Precision

Sample	Mean U/mL (n = 25)	CV (%)
1	3.9	7.7
2	7.0	8.6
3	28	3.2
4	58	3.1

10.5 Inter Assay Precision

Sample	Mean U/mL (n =2 0)	CV (%)
A	5.0	15.6
B	13.3	10.5
C	35	7.9
D	59	7.5

10.6 Clinical Accuracy

Analysis of 205 sera from patients with autoimmune diseases other than neuromyelitis optica spectrum disorders (NMOSD) indicated no interference from autoantibodies to the TSH receptor (n=110), glutamic acid decarboxylase (n=26), 21-hydroxylase (n=12), the acetylcholine receptor (n=10), thyroid peroxidase (n=15), thyroglobulin (n=10), IA-2 (n=7) or from rheumatoid factor (n=15) in the Aquaporin-4 Autoantibody ELISA (EIA-5431).

10.7 Interference

No interference was observed when samples were spiked with the following materials; bilirubin at 20 mg/dL or intralipid up to 3000 mg/dL. Interference was seen from haemoglobin at 500 mg/dL.

11 SAFETY CONSIDERATIONS**Streptavidin Peroxidase (SA-POD)****Signal word:** Warning**Hazard statement(s)**

H317: May cause an allergic skin reaction

Precautionary statement(s)

P280: Wear protective gloves/protective clothing/ eye protection/face protection

P302 + P352: IF ON SKIN: Wash with plenty of soap and water

P333 + P313: If skin irritation or rash occurs: Get medical advice/attention

P362 + P364: Take off contaminated clothing and wash it before reuse

Peroxidase Substrate (TMB)**Signal word:** Danger**Hazard statement(s)**

H360: May damage fertility or the unborn child

Precautionary statement(s)

P280: Wear protective gloves/protective clothing/ eye protection/face protection

P308 + P313: IF exposed or concerned: Get medical advice/attention

Diluent for SA-POD**Hazard statement(s)**

EUH208: Contains 2-Chloroacetamide. May produce an allergic reaction.

This kit is intended for use by professional persons only.

Follow the instructions carefully.

Observe expiry dates stated on the labels and the specified stability for reconstituted reagents.

Refer to Safety Data Sheet for more detailed safety information.

Avoid all actions likely to lead to ingestion. Avoid contact with skin and clothing. Wear protective clothing.

Material of human origin used in the preparation of the kit has been tested and found non-reactive for HIV1 and 2 and HCV antibodies and HBsAg but should, none-the-less, be handled as potentially infectious.

Wash hands thoroughly if contamination has occurred and before leaving the laboratory.

Sterilise all potentially contaminated waste, including test specimens before disposal.

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.

Some components contain small quantities of sodium azide as preservative. With all kit components, avoid ingestion, inhalation, injection or contact with skin, eyes or clothing. Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.

12 ASSAY PLAN













Allow all reagents and samples to reach room temperature (20 °C - 25 °C) before use	
Pipette:	50 µL Calibrators, controls and patient sera
Pipette:	25 µL AQP4-Biotin (reconstituted) into each well (except blank)
Incubate:	2 Hours at room temperature on an ELISA plate shaker at 500 shakes/min
Aspirate/Decant:	Plate
Wash:	Plate three times and tap dry on absorbent material ¹
Pipette:	100 µL SA-POD (diluted 1:20) into each well (except blank)
Incubate:	20 Minutes at room temperature on a ELISA plate shaker at 500 shakes/min
Aspirate/Decant:	Plate
Wash:	Plate three times and tap dry on absorbent material ^{1,2}
Pipette:	100 µL TMB into each well (including blank)
Incubate:	20 Minutes at room temperature in the dark without shaking
Pipette:	100 µL Stop solution into each well (including blank) and shake for 5 seconds
Read absorbance at 450 nm and 405 nm within 10 minutes of adding stop solution ³	

¹It is not necessary to tap the plates dry after washing when an automatic plate washer is used

²Use pure water for the final wash when washing manually

³If it is possible to read at only one wavelength, 405 nm may be used

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français	Português
	European Conformity	CE-Konformitätskennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes	Conformidade Europeia
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation	Consultar as instruções de uso
	<i>In vitro</i> diagnostic medical device *	<i>In-vitro</i> -Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro	Dispositivo médico para diagnóstico in vitro
	Catalogue number *	Katalognummer *	No. di Cat.	No de catálogo	Référence	Número de catálogo
	Batch code *	Chargen-bezeichnung *	Lotto no	Número de lote	No. de lot	Código do lote
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests	Suficiente para <n> determinações
	Temperature limit *	Temperaturgrenzwerte *	Temperatura di conservazione	Temperatura de conservación	Temperature de conservation	Limites de temperatura
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation	Prazo de validade
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant	Fabricante
	Distributor *	Vertriebspartner *	Distributore	Distribuidor	Distributeur	Distribuidor
	Date of manufacture *	Herstellungsdatum *	Data di produzione	Fecha de fabricación	Date de production	Data de fabricação
	Biological risks *	Biologische Risiken *	Rischi biologici	Riesgos biológicos	Risques biologiques	Riscos biológicos
	Caution *	Achtung *	Attenzione	Precaución	Attention	Cuidado
	Unique device Identifier *	eindeutige Produktidentifizierung *	Identificativo unico del dispositivo*	Identificación exclusiva del dispositivo *	Identifiant de dispositif unique*	Identificador único do dispositivo *
	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches	
<i>Content</i>	Content	Inhalt	Contenuto	Contenido	Conditionnement	Conteúdo
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité	Volume / Quantidade