



Revised 17 Jan. 2011 rm (Vers. 3.1)

USA: 

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The Acetylcholine Receptor Autoantibody (AChRAb) ELISA kit is intended for use by professional persons only, for the determination of AChRAb in human serum.

2 REFERENCES / LITERATURE

R. Hower et al, A sensitive non-isotopic assay for acetylcholine receptor autoantibodies, Clinica Chimica Acta 2006 **364**: 159 – 166

3 ASSAY PRINCIPLE

The AChRAb ELISA depends on the ability of AChRAb in human serum to bind to similar sites on the AChR as various monoclonal antibodies such as MAb1 (coated on ELISA plate wells) and/or MAb2 and/or MAb3 (which are labelled with biotin). In the absence of AChRAb a complex is formed between MAb1 coated on the plate wells, the AChR and MAb2 and MAb3 biotin. MAb2 and MAb3 biotin bound are then detected by addition of streptavidin peroxidase (SAPOD), substrate (TMB) and stop solution.

In the presence of AChRAb the formation of the MAb1-AChR-MAb2/MAb3 biotin complex is inhibited, resulting in less SAPOD being bound and a reduction in final absorbance at 450 nm.

The higher the concentration of AChRAb in the test serum, the greater the inhibition of MAb biotin binding.

When using the kit calibrators, the measuring range is 0.45 – 20 nmol/L toxin bound.

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 determinations).

Repeated freeze thawing or increases in storage temperature must be avoided.

Do not use lipaemic or haemolysed serum samples.

Do not use plasma in the assay.

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 g in a microfuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.



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5 MATERIALS REQUIRED AND NOT SUPPLIED

Pipettes capable of dispensing 25 µL, 50 µL and 100 µL.

Eppendorf type repeating pipette

Means of measuring various volumes to reconstitute or dilute reagents supplied

Eppendorf tubes

Pure water

ELISA plate reader suitable for 96 well formats and capable of measuring at 450 nm.

ELISA Plate shaker, capable of 500 shakes/min (not an orbital shaker).

ELISA Plate cover.

6 PREPARATION OF REAGENTS SUPPLIED

A	<i>Foetal type AChR , 3 x 0.7 mL Lyophilised</i>
	<i>Reconstitute each vial with 0.7 mL buffer for AChR (C). Mix gently, and leave to stand at room temperature for 5 minutes before use. Pool the vials when more than one vial is required. Use on day of reconstitution.</i>
B	<i>Adult type AChR , 3 x 0.5 mL Lyophilised</i>
A+B	<i>Reconstitute each vial of B with 0.5 mL of the solution of reconstituted foetal type AChR (A) to give a mixture of foetal and adult AChR (A+B). Mix gently, and leave to stand at room temperature for 5 minutes before use. Pool the vials when more than one vial is required. Use on day of reconstitution.</i>
C	<i>Buffer for AChR , 1 x 5 mL Ready for use</i>
D	<i>MAB1 Coated Wells 12 break apart strips of 8 wells (96 in total) in a frame and sealed in foil bag. Allow foil bag to stand at room temperature for 30 minutes before opening.</i>
	<i>Ensure strip wells are firmly fitted in the frame provided. After opening return any unused wells to the original foil packet and seal with adhesive tape. Then place foil bag in the self-seal plastic bag with desiccant provided, and store at 2 – 8°C for up to 16 weeks.</i>
E1-4	<i>Calibrators, 4 x 0.7 mL 0.5, 1.0, 6.5 and 20 nmol/L toxin bound Ready for use</i>
F1-2	<i>Positive controls, 2 x 0.7 mL (see label for concentration range) Ready for use</i>
G	<i>Negative control, 3.0 mL Ready for use</i>
H	<i>MAB–biotin (MAB2+MAB3) , 3 vials Lyophilised</i>



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	Reconstitute each vial with the volume of buffer for biotin (I) shown on the vial label. Mix gently, and leave to stand at room temperature for 5 minutes before use. Pool the vials when more than one vial is required. Store at 2 – 8°C for up to 16 weeks after reconstitution.
I	Buffer for MAb-biotin, 15 mL Ready for use
J	Streptavidin Peroxidase (SAPOD), 1 x 0.7 mL Concentrated Dilute 1 in 20 with diluent for diluting SAPOD (K). For example, 0.5 mL (J) + 9.5 mL (K). Store at 2 – 8°C for up to 16 weeks after reconstitution.
K	Diluent for SAPOD, 15 mL Ready for use
L	Peroxidase Substrate (TMB), 15 mL Ready for use
M	Concentrated wash solution, 100 mL Concentrated Dilute 1 in 10 with pure water before use. Use up to expiry of kit.
N	Stop solution, 10 mL Ready for use



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ASSAY PROCEDURE

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

An Eppendorf type repeating pipette is recommended for steps 2, 5, 7, 9 and 10.

DAY ONE	1.	Pipette 100 µL of samples (calibrators (E1-4 – optional), positive controls (F1-2) and negative control (G) and test sera) into individual 1.5 mL Eppendorf tubes, labelled accordingly.
	2.	Pipette 25 µL of foetal and adult type AChR mix (A+B) into each Eppendorf tube (from step 1) and seal the tubes. Make sure that all liquid is in the bottom of each tube (if in doubt centrifuge the tubes in a microfuge for 10 seconds at 10–15,000g). Vortex gently and incubate overnight (16-20 hrs) at 2 – 8°C.
DAY TWO	3.	Gently mix each tube of sample-AChR mixture from step 2 using a vortex mixer. Pipette duplicate 50 µL of each sample-AChR mixture into the ELISA plate wells (D) leaving 2 wells empty for blanks. Cover the wells and incubate at room temperature on an ELISA plate shaker (500 shakes per min) for one hour.
	4.	Aspirate the plate wells by use of a plate washing machine or discard by briskly inverting the frame of strip wells over a suitable receptacle. Wash the wells three times with diluted wash solution (M). Tap the inverted wells gently on a clean dry absorbent surface to remove excess wash.
	5.	Pipette 50 µL of reconstituted MAb-biotin (H) into each well (except blanks). Cover the plate and incubate at room temperature on an ELISA plate shaker (500 shakes per min) for one hour.
	6.	Repeat wash step 4.
	7.	Pipette 100 µL of diluted SAPOD (J) into each well (except blanks). Cover the plate and incubate at room temperature on an ELISA plate shaker (500 shakes per min) for 30 minutes.
	8.	Aspirate the plate wells by use of a plate washing machine or discard by briskly inverting the frame of strip wells over a suitable receptacle. Wash the wells three times with diluted wash solution (M), followed by once with pure water to remove any foam (if a plate washing machine is used, omit this water wash step). Tap the inverted wells gently on a clean dry absorbent surface to remove excess wash.
	9.	Pipette 100 µL of TMB (L) into each well (except blanks) and incubate in the dark at room temperature for 30 minutes without shaking.
	10.	Pipette 50 µL stop solution (N) to each well and shake the plate for approximately 5 seconds on a plate shaker. Ensure substrate incubations are the same for each well.
	11.	Read the absorbance of each well at 450 nm using an ELISA plate reader, blanked against a well containing 100 µL of TMB substrate (L) and 50 µL Stop solution (N) only.

7 RESULT ANALYSIS

A calibration curve can be established by plotting calibrator concentration on the x-axis (linear scale) against the absorbance of the calibrators on the y-axis (linear scale). A 4 parameter curve fit is recommended.

The AChR autoantibody concentrations in sample sera can then be read off the calibration curve.

We suggest assigning a value of 0.2 nmol/L toxin bound* for the kit negative control when using the calibrator curve.

Other data reduction systems can be used. For example, a point to point curve fit plotting calibration concentration on the x-axis (log scale) against the absorbance of calibrators on the y-axis (linear scale).

Samples with high AChRAb concentrations can be diluted in kit negative control (G).

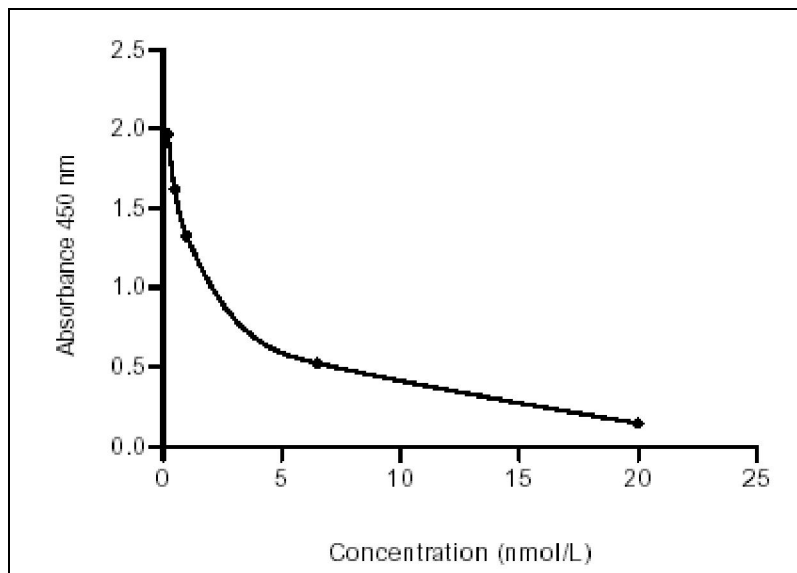
For example 10 µL of sample plus 90 µL of negative control to give a 10x dilution. Other dilutions (e.g. 100x) can be prepared from a 10x dilution or otherwise as appropriate.

Some sera will not dilute in a linear way and we suggest that the dilution giving a value closest to 50% inhibition is used for calculation of AChRAb concentration.

TYPICAL RESULTS WITH THE STANDARD CURVE

(Example only, not for calculation of actual results)

	Abs. 450 nm	Conc. nmol/L
Negative Control G	1.970	0.2*
E1	1.616	0.5
E2	1.329	1.0
E3	0.524	6.5
E4	0.144	20
Positive Control F1	0.469	7.5
Positive Control F2	1.124	1.6



Results can also be expressed as inhibition (%I) of AChR binding calculated using the formula;

$$100 \times \left\{ 1 - \frac{\text{Test sample absorbance at 450 nm}}{\text{Negative control absorbance (G) at 450 nm}} \right\}$$

This % inhibition value can then be converted to nmol/L toxin bound using the formula;

$$0.2 \times 2 (0.067 \times \% \text{ Inhibition of test sample})$$

This formula has been established empirically using a comparison of AChRab measurements by this ELISA and RIA methods.

8 ASSAY PLAN

DAY ONE	Allow all reagents and samples to reach room temperature (20 - 25 °C) before use	
	Pipette:	100 µL calibrators (optional) and positive and negative controls and test sera.
	Pipette:	25 µL AChR (foetal and adult mix) (centrifuge if necessary) and vortex mix.
	Incubate:	16 – 20 hours at 2 – 8°C.
DAY TWO	Pipette:	50 µL in duplicate of sample-AChR mixture from each tube into ELISA plate wells (except blanks).
	Incubate:	1 hour 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.
	Pipette:	50 µL MAb biotin into each well (except blanks).
	Incubate:	1 hour 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.
	Pipette:	100 µL SAPOD into each well (except blanks).
	Incubate:	30 minutes at room temperature on a ELISA plate shaker at 500 shakes/min.
	Aspirate/Decant:	Plate
	Wash:	Plate three times and rinse with pure water ¹ and tap dry on absorbent material.
	Pipette:	100µL TMB into each well (including blanks)
	Incubate:	30 minutes <u>in the dark</u> at room temperature without shaking.
	Pipette:	50 µL stop solution into each well (including blanks) and shake for 5 seconds.



DRG[®] Acetylcholine Receptor Autoantibody (AChRAb) ELISA (EIA-4936)



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	Read absorbance at 450 nm
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1) Omit water wash if a plate washing machine is used.