

**Acetylcholine Receptor Autoantibody (AChRab) RIA Kit - Instructions for use****RSR Limited**

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INTENDED USE

The RSR Acetylcholine Receptor autoantibody (AChRab) kit is intended for use by professional persons only for the quantitative determination of acetylcholine receptor (AChR) autoantibodies in human serum. Autoantibodies to the AChR are responsible for failure of the neuromuscular junction in myasthenia gravis. Measurement of the antibodies can be of considerable value in disease diagnosis.

REFERENCES

K Ohta et al

Frequency of Anti AChR ϵ subunit-specific antibodies in MG.

Autoimmunity 2003 36: 151-154

I. Matthews et al

Muscle-specific receptor tyrosine kinase autoantibodies – a new immunoprecipitation assay. Clinica Chimica Acta 2004 348: 95-99

D. Beeson et al

A transfected human muscle cell line expressing the adult subtype of the human muscle acetylcholine receptor for diagnostic assays in myasthenia gravis. Neurology 1996 47: 1552-1555

ASSAY PRINCIPLE

Adult and foetal forms of the acetylcholine receptor differ by one of their subunits (the gamma subunit in foetal receptor is replaced by the epsilon subunit in adult receptor). Furthermore AChRab in some sera recognise the foetal form of the receptor preferentially whereas AChRab in other sera recognise the adult form of the receptor preferentially. Consequently, a carefully balanced mixture of detergent solubilised foetal and adult forms of the receptor is the optimum preparation for AChRab assays. This mixture of receptors, labelled with ¹²⁵I-labelled alpha bungarotoxin provides the basis for RSR's AChRab assay kit. In the assay labelled receptors (¹²⁵I-AChR) are incubated with test sera and any resulting complex

of labelled receptor and receptor antibody immunoprecipitated with anti human IgG. After centrifugation and a wash step, the precipitate is counted.

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. Duplicate 5 μ L determinations are sufficient for one assay. Repeated freeze thawing or increases in storage temperature must be avoided. Incorrect storage of serum samples can lead to loss of antibody activity. Do not use lipaemic or haemolysed serum samples. Plasma may be used if EDTA has been used as the anticoagulant. 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 any particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

SYMBOLS

Symbol	Meaning
	EC Declaration of Conformity
	In Vitro Diagnostic Device
	Catalogue Number
	Lot Number
	Consult Instructions
	Manufactured by
	Sufficient for
	Expiry Date
	Store
	Positive Control
	Negative Control

MATERIALS SUPPLIED IN 25, 50 AND 100 TUBE KITS

MATERIAL	25 Tube	50 Tube	100 Tube
¹²⁵ I AChR	1 x 1.3mL	2 x 1.3mL	4 x 1.3mL
Negative Control	1 x 100 μ L	1 x 100 μ L	1 x 100 μ L
Positive Control	1 x 100 μ L	1 x 100 μ L	1 x 100 μ L
Anti Human IgG	1 x 1.5mL	2 x 1.5mL	1 x 5.5mL

Normal Human Serum	1 x 1mL	2 x 1mL	1 x 4mL
Washing Solution	1 x 60mL	2 x 60mL	2 x 120mL
Reconstitution Buffer	1 x 4mL	2 x 4mL	4 x 4mL
Precipitation Enhancer (optional)	1 x 1mL	2 x 1mL	4 x 1mL

MATERIALS REQUIRED AND NOT SUPPLIED

3.5 mL assay tubes (round bottomed tubes are recommended when using precipitation enhancer)

Suitable rack for assay tubes

Calibrated pipettes capable of dispensing 5 μ L, 25 μ L, 50 μ L, 100 μ L and 1 mL

Refrigerated centrifuge capable of 1500 g

Vortex mixer

Suitable apparatus for aspirating assay tubes

Gamma counter

PREPARATION OF REAGENTS SUPPLIED

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

A	¹²⁵I Labelled AChR Lyophilised	40kBq/vial (at manufacture)
	Reconstitute each vial with 1.3 mL of reconstitution buffer (G) and mix gently to dissolve. Once reconstituted, store at 2-8°C for up to 2 weeks. Mix thoroughly immediately before use.	
B	Negative Control	Ready for use
C	Positive Control (see QC sheet for concentration range)	Ready for use
D	Anti Human IgG	Ready for use
E	Normal Human Serum	Ready for use
F	Wash Solution	Ready for use
G	Reconstitution Buffer	Ready for use
H	Precipitation Enhancer (optional)	Ready for use
	Mix thoroughly immediately before use	

ASSAY PROCEDURE

Allow all reagents, excluding wash solution, to stand at room temperature (20-25°C) for at least 30 minutes prior to start of assay. A repeating Eppendorf type pipette is recommended for steps 2, 5, 8, 9 and 12.

1	Pipette 5 μ L of kit controls (B, C) and patient sera (undiluted), into labelled assay tubes in duplicate (calibrated pipettes will improve inter-assay precision).
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2	Pipette 50 μ L of ¹²⁵ I AChR (A) into each tube.
3	Mix tube contents by use of a vortex mixer and cover the tubes with a suitable cover.
4	Incubate at room temperature for 2 hours. During this incubation count 2 tubes for 3 seconds to determine total counts.
5	Pipette 50 μ L of anti human IgG (D) into each tube.
6	Repeat step 3.
7	Incubate at 2-8°C for 2 hours (an overnight incubation of 17-21 hours at 2-8°C can be used if necessary).
8	Pipette 25 μ L of precipitation enhancer (H) into each tube (optional).
9	Pipette 1 mL cold wash solution (F) into each tube and mix the contents using a vortex mixer.
10	Centrifuge each tube for 20 minutes at 2-8°C at 1500 g.
11	Aspirate or decant the supernatant.
12	Pipette 1 mL cold wash solution (F) into each tube and resuspend the pellet using a vortex mixer.
13	Repeat centrifugation step 10.
14	Aspirate or decant the supernatant
15	Count each tube for ¹²⁵ I for 2 minutes in a gamma counter.

RESULT ANALYSIS

The radioactivity in the final pellet is proportional to the amount of labelled AChR bound by AChRAB. This can be expressed as nmol of labelled AChR bound per litre of test serum using the following equation:

$$\text{nmol/L AChR bound} = \frac{(\text{cpm test sample} - \text{cpm negative control}) \times A}{C \times K \times B \times 2.22}$$

where;

A is the decay factor for ¹²⁵I between the receptor labelling day and the day of assay; **B** is the counter efficiency; **C** is the volume of serum used in the assay (μ L) and **K** is the specific activity (Ci/mmol) of the ¹²⁵I-labelled toxin at the time it was used to label the AChR. Values for A, C and K are provided with each kit lot on a separate sheet.

TYPICAL RESULTS (example only, not for calculation of actual results)

Total counts 77,120	Mean counts per min in pellet	Conc. nmol/L
Test sample		
Kit negative control (B)	608	
Kit positive control (C)	10680	4.4
Patient serum 1	6502	2.6
Patient serum 2	2202	0.7
Patient serum 3	15555	6.6

Example Calculation

If a test sample counts are 2756 cpm, negative control counts are 608 cpm and the assay was carried out with 5 µL of test serum, 2 weeks after the labelling date using toxin with a specific activity of 216 Ci/mmole and with a counter efficiency of 0.7, then A = 1.2, B = 0.7, C = 5 and K = 216.

$$\text{AChRab Conc.} = \frac{(2756 - 608) \times 1.2}{5 \times 216 \times 0.7 \times 2.22} = 1.5 \text{ nmol/L}$$

Assay Calibrators

As an alternative to using the calculation described above a set of calibrators (available from RSR: nominally 0.25, 1, 4 and 8 nmol/L; see standard curve quality control record sheet for actual concentrations:) can be run in each assay and AChRab concentration read off a calibration curve. The calibrator concentrations provided are specific for each kit lot.

Assay Linearity

The relationship between acetylcholine receptor antibody concentration and cpm bound in the assay is only linear over a limited range. To overcome this problem, antibody positive sera can be diluted several times in the normal human serum (E) provided and assayed. Antibody concentrations can then be calculated using binding data from within the linear range. The linear range for different patient sera is often different but 0.5 – 5 nmol/L is a useful guide. For example, we can consider a test serum sample which gives a value of 9 nmol/L undiluted. Three fold, 9 fold and 27 fold dilutions give values of 12, 13 and 13 nmol/L respectively (after correction for the dilution factor), all 3 dilution values being clearly in the linear range as they agree well. The result for this sample is then expressed as the mean of these 3 values i.e. 12.7 nmol/L.

ASSAY CUT OFF

Cut off	nmol/L
Negative	< 0.5 nmol/L
Positive	≥ 0.5 nmol/L

CLINICAL EVALUATION

Clinical Specificity

112 samples from healthy blood donors were assayed in the AChRab RIA. 110 (98%) were identified as being negative for AChRab.

Clinical Sensitivity

Samples from 53 patients diagnosed with myasthenia gravis were assayed in the AChRab RIA and all 53 were identified as being positive for AChRab. In a larger series, K. Ohta et al (Autoimmunity 2003 36 151-154) found 82% of 1740 patients with myasthenia gravis to be AChRab positive using the RSR AChRab kit.

Lower Detection Limit

The AChRab RIA kit negative control was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at +2 standard deviations was 0.02 nmol/L.

Inter Assay Precision

Sample	Mean nmol/L (n = 21)	CV (%)
1	2.2	5.0
2	0.5	5.9

Intra Assay Precision

Sample	Mean nmol/L (n = 20)	CV (%)
1	3.3	1.9
2	1.8	1.7.

Clinical Accuracy

No interference from autoantibodies to TSH receptor, thyroglobulin, thyroid peroxidase, GAD, 21-OH, double stranded DNA or from rheumatoid factor was detected using the AChRab kit.

Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 5 mg/mL, 20 mg/dL bilirubin or Intralipid up to 30 mg/dL.

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for AChRab levels.

SAFETY CONSIDERATIONS

Precipitation Enhancer

Signal word: Warning



Hazard statement(s)

H373: May cause damage to organs through prolonged or repeated exposure

Precautionary statement(s)

P260: Do not breathe dust/fume/gas/mist/vapours/spray

P314: Get medical advice/attention if you feel unwell

Follow the instructions carefully. Observe expiry dates stated on the labels and the specified stability of reconstituted reagents. Refer to Material Safety Data Sheet for more detailed safety information. The kit contains radioactive material. Users should make themselves aware of and observe any national and local legislation and codes of practice governing the use, storage, transportation and disposal of radioactive materials. Avoid all actions likely to lead to ingestion. Avoid contact with skin and clothing. Wear protective clothing and where appropriate personal

dosimeters. Radioactive materials should only be used by authorised personnel and in designated areas. Wash hands thoroughly after handling. Monitor hands and clothing before leaving the designated area.

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 this material should none-the-less be handled as potentially infectious. Some components contain small quantities of sodium azide as preservative. With all kit components, avoid ingestion, inhalation, injection and contact with skin, eyes and clothing. Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.

ASSAY PLAN

Allow all reagents (excluding wash solution) and samples to reach room temperature (20–25 °C) before use.	
Pipette:	5 µL kit controls (B, C) and patient serum (undiluted)
Pipette:	50 µL ¹²⁵ I AChR (A)
Tubes:	Mix on vortex mixer and cover
Incubate:	2 hours at room temperature. Count 2 tubes for 3 seconds for total counts.
Pipette:	50 µL of anti human IgG (D) into all tubes
Tubes:	Mix on vortex mixer and cover
Incubate:	2 hours at 2–8°C (or overnight for 17–21 hours at 2–8°C)
Pipette:	25 µL precipitation enhancer (H) (optional)
Pipette:	1 mL cold wash solution (F)
Tubes:	Mix on vortex mixer
Tubes:	Centrifuge each tube for 20 minutes at 2–8°C at 1500 g
Tubes:	Aspirate/Decant supernatant
Pipette:	1 mL cold wash solution (F)
Tubes:	Mix on vortex mixer to resuspend pellet
Tubes:	Centrifuge each tube for 20 minutes at 2–8°C at 1500 g
Tubes:	Aspirate/Decant supernatant
Count tubes for ¹²⁵ I for 2 minutes using gamma counter	