RSR

RiaRSR™ gAChR Ab

Ganglionic Acetylcholine Receptor (gAChR) Autoantibody RIA Kit - Instructions for use

FOR RESEARCH USE ONLY

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INTENDED USE

The RSR Ganglionic Acetylcholine Receptor (gAChR) autoantibody (Ab) RIA kit is intended for use by professional persons only, for the quantitative determination of gAChR Abs in human serum. Serum autoantibodies reactive with gAChR are implicated in impaired synaptic transmission at autonomic ganglia, specifically associated with Autoimmune Autonomic Ganglionopathy (AAG) and gastro-intestinal dysmotility. Functional gAChRs are pentameric, consisting of α3 and β4 subunits and are expressed predominately in autonomic ganglia. gAChR Abs primarily bind to the α3-subunit. The kit is easy to use and provides a specific and sensitive assay for gAChR Ab.

REFERENCES

Vernino et al
Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies.
N. Engl. J. Med. 343 (2003) 847 – 855

McKeon et al
The ganglionic acetylcholine receptor autoantibody: oncological, neurological and serological accompaniments.
Arch. Neurol. 66 (2009) 735 – 741

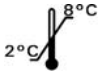
ASSAY PRINCIPLE

The assay depends on the use of recombinant gAChR complexed with ^{125}I-labelled epibatidine. The ^{125}I-labelled gAChRs are then incubated with test sera and the resulting complexes immunoprecipitated with anti-human IgG. The higher the concentration of autoantibody, the greater the amount of radioactivity precipitated.

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. 20 µL is sufficient for one assay (duplicate 10 µL determinations). Repeated freeze thawing or increases in storage temperature must be avoided. Do not use lipaemic or haemolysed serum samples. On the day of assay, thaw the sera at room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to assay (preferably for 5 min at about 10,000 rpm i.e. about 10,000g 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
RUO	For Research Use Only
REF	Catalogue Number
LOT	Lot Number
	Consult Instructions
	Manufactured by
	Sufficient for
	Expiry Date
	Store
CONTROL -	Negative Control
CONTROL +	Positive Control

MATERIALS REQUIRED AND NOT SUPPLIED

4.5 mL Conical plastic tubes
Pipettes capable of dispensing 10 µL, 50 µL, 0.75 mL and 1 mL
Vortex mixer
Refrigerated centrifuge capable of 1500g
Gamma counter

PREPARATION OF REAGENTS SUPPLIED FOR 25 TUBE KIT

Store unopened kits and all components at 2 – 8°C.

A	**^{125}I Labelled gAChR** ~6kBq/vial (at manufacture) 2 vials Lyophilised
	Reconstitute each vial by addition of 0.75 mL reconstitution buffer (B) and mix gently to dissolve. **Use immediately.**
B	**Reconstitution Buffer for ^{125}I Labelled gAChR** 2 mL Ready for use
C	**Negative Control** 0.1 mL Ready for use

D 1-2	**Positive Controls I & II** 2 x 0.1 mL Ready for use. See vial label for concentration range
E	**Anti Human IgG** 2 mL Ready for use
F	**Wash Solution** 60 mL Ready for use and keep at 2 – 8°C except when in use.

ASSAY PROCEDURE

Allow all reagents, **except wash solution**, to stand at room temperature (20 – 25°C) for at least 30 minutes before use. An Eppendorf type repeating pipette is recommended for steps 2, 4, 6, and 9.

1.	Pipette **10 µL** (in duplicate) of negative control (C), positive controls (D1-2) and patient sera (all undiluted), into labelled conical assay tubes.
2.	Pipette 50 µL of **freshly reconstituted** ^{125}I labelled gAChR (A + B) into each tube and into two additional empty tubes for total counts.
3.	Mix each tube gently on a vortex mixer; cover the tubes with a suitable cover and incubate at room temperature (20 – 25°C) for 2 hours.
4.	Pipette 50 µL of anti human IgG (E) into each tube (excluding the two total count tubes).
5.	Mix each tube gently on a vortex mixer; cover the tubes with a suitable cover and incubate at room temperature (20 – 25°C) for 2 hours.
6.	Pipette 1 mL of **cold** (2 – 8°C) wash solution (F) into each tube (excluding the two total count tubes) and mix gently on a vortex mixer.
7.	Centrifuge each tube at 1500g for 20 minutes at 2 – 8°C.
8.	Aspirate or decant the supernatant.
9.	Pipette 1 mL of **cold** (2 – 8°C) wash solution (F) into each tube (excluding the two total count tubes) and resuspend the pellet gently using a vortex mixer.
10.	Repeat steps 7 and 8.
11.	Count each tube (including total count tubes) for 2 minutes using a gamma counter.

RESULT ANALYSIS

The radioactivity in the pellet represents the amount of ^{125}I-labelled gAChR bound by the gAChR Abs. This is often expressed as picomoles of labelled toxin bound per litre of test serum and the relationship between this parameter and pellet radioactivity can be calculated using the following equation:

$$\text{pmol / L} = \frac{(\text{cpm test sample} - \text{cpm negative control}) \times 1000 \times A}{C \times K \times B \times 2.22}$$

where; **A** is the decay factor for ^{125}I between the tracer manufacture date and the day of the assay (as provided in QC sheet); **B** is the counter efficiency; **C** is the volume of serum used in the assay (i.e. 10 µL) and **K** is the specific activity (Ci/mmol) of the ^{125}I-labelled toxin at the time it was used to label gAChRs, (as provided in QC sheet).

TYPICAL RESULTS (example only; not for use in calculation of actual results)

	cpm	pmol/L
Negative Control	608	0.0
Positive Control I	4844	134.2
Positive Control II	1505	28.4

ASSAY CUT OFF

Negative	< 10 pmol/L
Indeterminate	between 10 and 15 pmol/L
Positive	≥ 15 pmol/L

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

CLINICAL EVALUATION

Clinical Specificity

Sera from 50 individual healthy blood donors were assayed in the gAChR Ab RIA. 50 (100%) were identified as being negative for gAChR Ab.

Clinical Accuracy

Sera from 30 patients with autoimmune diseases other than those with suspected AAG and related neurological disorders were assayed in the gAChR Ab RIA. 29 (96.7%) of GAD or TSH receptor autoantibody-positive samples tested negative for gAChR Ab in this study, with 1 further sample (3.3%) proving indeterminate.

SAFETY CONSIDERATIONS

This kit is intended for *in vitro* use by professional persons only. Follow the instructions carefully. Observe expiry dates stated on the labels and the specified shelf life for reconstituted reagents. Refer to 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. Materials of human origin used in the preparation of the kit have 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. Materials of animal origin used in the preparation of the kit have 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.

ASSAY PLAN

Allow all reagents, **except wash solution**, to stand at room temperature (20-25°C) for at least 30 minutes before use	
Pipette:	**10** µL negative control (C), positive controls (D1-2) and patient sera (all undiluted)
Pipette:	**50** µL ^{125}I labelled gAChR (A) (**freshly reconstituted (B)**) into all tubes plus two additional empty tubes for total counts
Mix:	Mix tubes gently on vortex mixer and cover
Incubate:	2 hours at room temperature (20 – 25°C)
Pipette:	**50** µL anti human IgG (E) into all tubes (excluding the two total count tubes)
Mix:	Mix tubes gently on vortex mixer and cover
Incubate:	2 hours at room temperature (20 – 25°C)
Pipette:	**1 mL cold** (2 – 8°C) wash solution (F) (excluding the two total count tubes)
Mix:	Mix tubes gently on vortex mixer
Centrifuge:	Centrifuge tubes at 1500g for 20 minutes at 2 – 8°C
Aspirate/Decant:	Aspirate or decant supernatants for tubes
Pipette:	**1 mL cold** (2 – 8°C) wash solution (F) (excluding the two total count tubes)
Mix:	Mix tubes on vortex mixer to resuspend pellet
Centrifuge:	Centrifuge tubes at 1500g for 20 minutes at 2 – 8°C
Aspirate/Decant:	Aspirate or decant supernatants for tubes
Count tubes for 2 minutes using gamma counter	