RSR

RiaRSR™N- VGCC Ab

N-Type Voltage-Gated Calcium Channel (N-VGCC) Autoantibody RIA Kit - Instructions for use

FOR RESEARCH USE ONLY

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INTENDED USE

The RSR N-type voltage-gated calcium channel (N-VGCC) autoantibody RIA kit is intended for use by professional persons only, for the quantitative determination of N-type VGCC autoantibodies (N-VGCC Ab) in human serum. Serum autoantibodies reactive with N-VGCC were reported initially in dysfunction of the neuromuscular junction specifically associated with Lambert-Eaton Myasthenic Syndrome (LEMS). Subsequently, N-VGCC Abs were detected in disorders of the central nervous system, including cerebellar degeneration and in paraneoplastic autoimmunity, particularly associated with Small Cell Lung Cancer (SCLC). Functional N-VGCCs consist of a pore-forming α1-subunit together with ancillary β, γ and α2/δ subunits and are expressed widely in the central and peripheral nervous systems. N-VGCC Abs primarily bind to the α1-subunit. The kit is easy to use and provides a specific and sensitive assay for N-type VGCC Ab and is designed to complement the RiaRSR™VGCC Ab RIA kit for the detection of autoantibodies against P/Q-type VGCCs.

REFERENCES

M. Motomura et al
An improved diagnostic assay for Lambert-Eaton myasthenic syndrome.
J. Neurol. Neurosurg. Psychiatry (1995) 58:85-87

V. A. Lennon et al
Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes.
N. Engl. J. Med. (1995) 332:1467-1474.

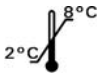
ASSAY PRINCIPLE

In RSR's N-VGCC Ab RIA, N-VGCC Ab in patient sera and controls are allowed to interact with detergent solubilised N-type VGCCs extracted from rabbit brain tissue and complexed with ^{125}I-labelled ω-conotoxin GVIA. After incubation at 2 – 8°C overnight, the resulting antigen-antibody complexes are immunoprecipitated by the addition of anti-human IgG. After a second incubation of 1½ hours, assay buffer is added and the samples centrifuged. Unbound ^{125}I-labelled ω-conotoxin GVIA is removed from the tubes by aspiration of the supernatant. The level of radioactivity remaining in the tube is proportional to the antibody level in the test sample.

STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at 2 – 8°C for up to 2 weeks, or at –20°C or below for longer periods. 15 µL is sufficient for one assay. Repeated freeze thawing or increases in storage temperature must be avoided. Do not use lipaemic or haemolysed serum samples. Citrate, EDTA and heparin plasma may be used in the assay. When required, thaw test sera at room temperature and mix gently to ensure homogeneity. Dilute 1:10 using assay buffer (e.g. 15 µL serum plus 135 µL assay buffer). Centrifuge diluted 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.

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 50 µL, 0.75 mL and 1 mL
Pure water
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 N-VGCC** **~15kBq/vial** 2 vials (at manufacture) Lyophilised
	Reconstitute each vial by addition of 0.75 mL pure water and vortex gently to dissolve. **Use immediately.**
B	**Negative Control** 0.25 mL Ready for use
C 1-2	**Positive Controls I & II** 2 x 0.25 mL Ready for use. See vial label for concentration range
D	**Anti-Human IgG** 2 mL Ready for use
E	**Assay Buffer** 60 mL Ready for use and keep at 2 – 8°C except when in use.

ASSAY PROCEDURE

Allow all reagents, **except assay buffe**r, 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 **50 µL** (in duplicate) of negative control (B), positive controls (C1-2) and diluted patient sera (diluted 1:10 in assay buffer), into labelled assay tubes (the controls are supplied ready diluted).
2.	Pipette 50 µL of **freshly reconstituted** ^{125}I-labelled N-VGCC (A) 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 2 – 8°C for 16 - 20 hours.
4.	Pipette 50 µL of anti-human IgG (D) 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 2 – 8°C for 1½ hours.
6.	Pipette 1 mL of **cold** (2 – 8°C) assay buffer (E) 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 4°C.
8.	Aspirate or decant the supernatant.
9.	Pipette 1 mL of **cold** (2 – 8°C) assay buffer (E) 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 1 minute using a gamma counter.

RESULT ANALYSIS

The radioactivity in the pellet represents the amount of ^{125}I-labelled ω-conotoxin GVIA bound by the N-VGCC Ab. 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 from the knowledge of (values for K and A are on the QC record sheet):

(1) the specific activity (K Ci/mmol) of the ^{125}I-labelled toxin at the time it was labelled;
(2) the decay of the ^{125}I in the labelled toxin–N-VGCC complex in the period between labelling and the day of the assay, (decay factor A);
(3) the volume of neat serum used in the assay (C µL) (C = 5 for 50 µL of the 1:10 diluted sample);
(4) the counter efficiency of the gamma counter used (B);

The formula is as follows:-

pmol/L = 1000 x (cpm test sample – cpm negative control) x A/(C x K x B x 2.22)

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

	cpm	pmol/L
Negative Control	1461	0
Positive Control I	10064	652
Positive Control II	3915	186

ASSAY CUT OFF

Negative	≤110 pmol/L
Positive	>110 pmol/L

This cut off has been validated at RSR. However each laboratory should establish its own normal and pathological reference ranges for N-VGCC 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 59 individual healthy blood donors were assayed in the N-VGCC Ab RIA. 58 (98.3%) were identified as being negative for N-VGCC Ab.

Clinical Sensitivity

Serum samples from 20 patients positive for P/Q-type VGCC Ab in the VGCC Ab RIA were assayed in the N-VGCC Ab RIA. 6 (30%) were positive for N-VGCC Ab.

Clinical Accuracy

None of 30 patients with autoimmune diseases other than those with suspected LEMS and related neurological disorders were positive for N-VGCC Ab. This study indicated no interference from autoantibodies to GAD or the TSH receptor in the RSR N-VGCC Ab RIA.

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 assay buffe**r, to stand at room temperature (20-25°C) for at least 30 minutes before use	
Pipette:	**50** µL negative control ((B) ready to use, do not dilute), positive controls ((C1-2) ready to use, do not dilute) and diluted patient sera (diluted 1:10 in assay buffer)
Pipette:	**50** µL ^{125}I-labelled N-VGCC (A) (**freshly reconstituted**) into all tubes plus two additional empty tubes for total counts
Tubes:	Mix gently on vortex mixer and cover
Incubate:	16 - 20 hours at 2 – 8°C
Pipette:	**50** µL anti-human IgG (D) into all tubes (excluding the two total count tubes)
Tubes:	Mix gently on vortex mixer and cover
Incubate:	1½ hours at 2 – 8°C
Pipette:	**1 mL cold** assay buffer (E) (excluding the two total count tubes)
Tubes:	Mix gently on vortex mixer
Tubes:	Centrifuge at 1500g for 20 minutes at 4°C
Tubes:	Aspirate or decant supernatants
Pipette:	**1 mL cold** assay buffer (E) (excluding the two total count tubes)
Tubes:	Mix on vortex mixer to resuspend pellet
Tubes:	Centrifuge at 1500g for 20 minutes at 4°C
Tubes:	Aspirate or decant supernatants
Count tubes for 1 minute using gamma counter	