RSR

RiaRSR™ VGKC Ab

Voltage-Gated Potassium Channel (VGKC) Autoantibody RIA Kit - Instructions for use

CE

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INTENDED USE

The RSR VGKC autoantibody RIA kit is intended for use by professional persons only, for the quantitative determination of VGKC autoantibodies (VGKC Ab) in human serum. Serum VGKC Ab have been detected in peripheral nervous system disease specifically associated with the clinical spectrum of acquired neuromyotonia (NMT) and cramp-fasciculation syndrome (CFS), and disorders of the central nervous system, including Morvan syndrome, epilepsy and limbic encephalitis (LE). Detection and measurement of VGKC Ab are useful in the diagnosis and management of autoimmune Voltage-Gated Potassium Channelopathies and related neurological disorders. The kit is easy to use and provides a specific and sensitive assay for VGKC Ab.

REFERENCES

I. Hart et al.
"Autoantibodies detected to expressed K^+ channels are implicated in Neuromyotonia."
Ann Neurol **41** (1997), 238 - 246

A. Vincent et al.
"Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis."
Brain **127** (2004), 701 - 712

K. Tan et al.
"Clinical spectrum of voltage-gated potassium channel autoimmunity."
Neurology **70** (2008), 1883 - 1890

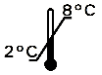
ASSAY PRINCIPLE

In RSR's VGKC Ab radioimmunoassay (RIA), VGKC Ab in patient sera and controls are allowed to interact with detergent solubilised VGKCs extracted from rabbit brain tissue and complexed with ^{125}I-labelled α-dendrotoxin (known to react with Kv1.1, 1.2 and 1.6 subtypes of the VGKC). 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 α-dendrotoxin-VGKC complex 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 10,000rpm i.e. about 10,000g in a microfuge) to remove any particulate matter.

SYMBOLS

Symbol	Meaning
CE	EC Declaration of Conformity
IVD	In Vitro Diagnostic Device
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

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

A	**^{125}I-Labelled VGKC** **~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	**Anti-Human IgG** 2 mL Ready for use
C	**Assay Buffer** 60 mL Ready for use and keep at 2-8 °C except when in use.
D	**Negative Control** 0.25 mL Ready for use
E 1-2	**Positive Controls I & II** 2 x 0.25 mL Ready for use. See vial label for concentration range

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 diluted patient sera (diluted 1:10 in assay buffer), negative control (D) and positive controls (E1-2) into labelled assay tubes (the controls are supplied ready diluted).
2.	Pipette 50 µL of **freshly reconstituted** ^{125}I-labelled VGKC (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 (B) 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 1½ hours.
6.	Pipette 1 mL of cold (2-8 °C) assay buffer (C) 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 (C) 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 α-dendrotoxin-VGKC complex bound by the VGKC 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 α-dendrotoxin-VGKC complex at the time it was labelled;
(2) the decay of the ^{125}I in the labelled α-dendrotoxin–VGKC 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 = 5µL for a 1:10 diluted sample);
(4) the counter efficiency of the gamma counter used (B);
(5) the cpm of the test sample or positive control minus the cpm of the negative control (D).

The formula is as follows:-

pmol/L = 1000 x D 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	2041	0
Positive Control I	10827	387
Positive Control II	4874	125

ASSAY CUT OFF

Negative	<72 pmol/L
Positive	≥72 pmol/L

CLINICAL EVALUATION

Clinical Specificity

Sera from 100 individual healthy blood donors were assayed in the VGKC Ab RIA. 98 (98%) were identified as being negative for VGKC Ab.

Clinical Sensitivity

Serum samples from 30 patients with suspected Voltage-Gated Potassium Channelopathies and related neurological disorders were assayed in the VGKC Ab RIA. 27 (90%) were positive for VGKC Ab.

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 4.5 pmol/L.

Intra Assay Precision

Sample	Mean pmol/L (n = 20)	CV (%)
1	102	5.7
2	150	5.8
3	332	3.7

Inter Assay Precision

Sample	Mean pmol/L (n = 12)	CV (%)
A	89	6.6
B	138	6.4
C	320	5.4

Clinical Accuracy

None of 138 patients with autoimmune diseases other than those with suspected Voltage-Gated Potassium Channelopathies and related neurological disorders were positive for VGKC Ab except for 1 (out of 17) patient with Type 1 Diabetes (IA-2 Ab positive) and 2 (out of 26) patients with Rheumatoid Arthritis. This study indicated no interference from autoantibodies to thyroglobulin, thyroid peroxidase, the TSH receptor, aquaporin-4, 21-hydroxylase, GAD and the acetylcholine receptor in the RSR VGKC Ab RIA kit.

Interference

No interference was observed when samples were spiked with the following materials; bilirubin up to 20 mg/dL, haemoglobin up to 500 mg/dL or intralipid up to 3000 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 VGKC Ab levels.

SAFETY CONSIDERATIONS

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. 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 Diluted patient sera (diluted 1:10 in assay buffer) negative and positive controls
Pipette:	50 µL ^{125}I-labelled VGKC (**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 into all tubes (excluding the two total count tubes)
Tubes:	Mix gently on vortex mixer and cover
Incubate:	1½ Hours at room temperature (20-25°C)
Pipette:	1 mL **Cold** assay buffer (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 (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	