DLD DIAGNOSTIKA GMBH

Instructions for Use

LEMS® N-type Assay RIA

^{125}I-Radio Immuno Assay
for the Quantitative Determination of Autoantibodies to N-type Voltage-gated Calcium Channel (N-VGCC) in Serum, Citrate, EDTA and Heparin Plasma

RUO

REF RA121/25

Σ 25

+2 +8 °C 2 – 8 °C

DLD Gesellschaft für Diagnostika und medizinische Geräte mbH
Adlerhorst 15 • 22459 Hamburg • Telefon: 040/ 555 87 10 • Fax: 040/ 555 87 111
Internet: http://www.dld-diagnostika.de • E-Mail: contact@dld-diagnostika.de

Contents

Symbols

RUO	Research Use Only		
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
REF	Catalogue number		Consult Instructions for Use

Hazard Pictograms

 Radioactive

1. Introduction and Principle of the Test

The LEMS® N-type Assay RIA kit is for the determination of N-type Voltage-gated Calcium Channel Autoantibodies (N-VGCC Ab) in human serum. The assay is intended for use by professional persons only and for research use only. Not for use in diagnostic procedures.

Serum autoantibodies reactive with N-VGCC were reported initially in dysfunction of the neuromuscular junction specially associated with Lambert Eaton Myasthenic syndrome (LEMS). Subsequently, N-VGCC Abs were detected in disorders of the central nervous system, including cerebellar degeneration and paraneoplastic autoimmunity, particularly associated with Small Cell Lung Cancer (SCLC). Functional N-VGGCs consist of a pore-forming $\alpha 1$-subunit together with ancillary β, γ and $\alpha 2/\delta$-subunits and are expressed widely in the central and peripheral nervous systems. N-VGCC Abs primarily bind to the $\alpha 1$-subunit. The kit is easy to use and provides a specific and sensitive assay for determination of N-type VGCC Ab and is designed to complement the LEMS® Assay RIA, which detects autoantibodies against P/Q-type VGCCs.

In the LEMS® N-type Assay RIA, N-VGCC autoantibodies in samples and controls are allowed to interact with detergent solubilised N-type VGCCs extracted from rabbit brain tissue and complexed with ^{125}I-labelled ω-contoxin 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 1.5 hours, assay buffer is added and the samples are 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.

2. Precautions

This kit is intended for *in vitro* use by professional persons only. Follow the instructions carefully. 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. It must not be administered to humans or animals under any circumstances. Avoid all actions likely to lead to ingestion; do not eat, drink or smoke where radioactive materials are being handled. Do not pipet by mouth. Avoid contact with skin and clothing. Wear protective clothing, disposable gloves, 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 copius amounts of water.

3. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened, the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the kit labels.

Do not mix various lots of any kit component within an individual assay.

4. Contents of the Kit

4.1. **^{125}I-labelled N-VCGGs** **TRACER**  2 vials

lyophilized, activity < 15 kBq/vial at manufacture

4.2. **Negative Control** **CON -** 1 vial

0.25 ml, ready for use

4.3. **Positive Control 1 & 2** **CON + I** **CON + II** 2 vials

0.25 ml each, ready for use,
for concentration ranges see QC Certificate

4.4. **Anti-human IgG** **ANTI-HUMAN-IGG** 1 vial

2 ml, ready for use

4.5. **Assay Buffer** **BUFFER** 1 bottle

60 ml, ready for use,
Keep at 2–8 °C except when in use

Additional materials and equipment required but not provided:

- Pipettes for dispensing 50 µl, 0.75 ml and 1 ml
- 4.5 ml conical plastic tubes and suitable rack
- Centrifuge capable of 2-8 °C and 1500 x g
- Dist. water
- Suitable device for aspirating or decanting the supernatant
- Vortex mixer
- Gamma counter

5. Specimen Collection and Storage

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. Repeated freezing and thawing should be avoided. Do not use lipemic or grossly haemolized serum. Citrate, EDTA and heparin plasma may be used in the assay.

6. Preparation of Samples and Reagents

6.1 Samples

When required, thaw test sera at room temperature and mix gently to ensure homogeneity.

Dilute sera 1:10, i.e. 1+9, with the Assay Buffer [BUFFER] (e.g. 15 µl serum + 135 µl Assay Buffer). Do not dilute negative and positive controls, as they are pre-diluted 1:10 and ready for use. Centrifuge diluted samples prior to assay (preferably for 5 min at 10.000 x g) to remove any particulate material.

6.2 Reconstitution of the Tracer

Approximately 10 min before use, reconstitute the lyophilized Tracers [TRACER] each with 0.75 ml distilled water. Dissolve by vortexing briefly (about 5 sec) on a vortex mixer and then keep the vial upright.

The reconstituted Tracers are stable for only a few hours and should be used at once.

7. Test Procedure

Allow all reagents, except Assay Buffer [BUFFER], to stand at room temperature (20 – 25 °C) for at least 30 minutes before use.

7.1. Pipette 50 µl (in duplicate) of the Negative Control [CON -], Positive Controls [CON + I] & [CON + II] and the 1:10-diluted samples, into labelled assay tubes (conical tubes recommended).

7.2. Add 50 µl of freshly reconstituted Tracer [TRACER] into each tube.

7.3. 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.

At the beginning of the incubation determine the total radioactivity for 1 minute in at least 2 tubes in a gamma counter.

7.4. Add 50 µl Anti-human IgG [ANTI-HUMAN-IGG] to each tube.

7.5. Mix each tube gently on a vortex mixer; cover the tubes with a suitable cover and incubate at 2 – 8 °C for 1.5 hours.

7.6. Add 1 ml of cold (2 – 8 °C) Assay Buffer [BUFFER] to each tube and mix gently on a vortex mixer.

7.7. Centrifuge tubes at 1.500 x g for 20 minutes at 4 °C.

7.8. Decant or aspirate the supernatant, taking care not to disturb the pellet.

7.9. Add 1 ml of cold (2 – 8 °C) Assay Buffer [BUFFER] to each tube and carefully resuspend the pellet using a vortex mixer.

7.10. Centrifuge all tubes at 1.500 x g for 20 minutes at 4 °C.

7.11. Decant or aspirate the supernatant, taking care not to disturb the pellet.

7.12. Determine the counts (cpm) in each tube for 1 minute in a gamma counter.

8. Calculation of Results

The radioactivity in the pellet represents the amount of ^{125}I-labbeled ω-conotoxin GVIA bound by the N-VGCC Ab. This is often expressed as picomoles of labelled toxin bound per liter of test serum and the relationship between this parameter and pellet radioactivity can be calculated from the knowledge of:

- Specific activity A [Ci/mmol] of the ^{125}I-labelled toxin at the time it was labelled.
- Decay factor D for the decay of ^{125}I in the labelled toxin-N-VGCC complex in the period between labelling and the day of the assay.
- The volume of neat serum used in the assay C (C = 5 µl for 50 µl of the 1:10 diluted sample: see Sample Preparation).
- Counter efficiency B of the gamma counter used (e.g. 70%, then B = 0.7)

Values for A and D are on the QC Certificate included in each kit.

The formula is a s follows:

$$\text{pmol/l} = \frac{1000 \times (\text{cpm}_{\text{Pos. Control OR Sample}} - \text{cpm}_{\text{Neg. Control}}) \times D}{C \times A \times B \times 2.22}$$

Typical Results (example only, not for use in calculation of actual results):

	cpm	**pmol/l**
Negative Control	1461	**0**
Positive Control 1	10064	**652**
Positive Control 2	3915	**186**

For assay to be valid, values achieved for both positive controls must be in target range indicated on QC certificate included in each kit.

9. Assay Characteristics

This kit is for research use only, the values below are not for use in diagnostic procedures.

The following assay cut off was determined:

Negative	≤ 110 pmol/l
Positive	> 110 pmol/l

However, each labaoratory should establish its own normal and pathological reference ranges for N-VGCC Ab levels. Also it is recommended that each labaoratory includes its own panel of control samples in the assay.

Sera from 59 individual healthy blood donors were assayed in the LEMS® N-VGCC Assay RIA. 58 (98.3%) were identified as being negative for N-type VGCC Ab.

Samples from 20 patients positive for P/Q-type VGCC Ab in the LEMS® Assay RIA were assayed in the LEMS® N-VGCC Assay RIA. Six (30%) were identified as being positive for N-type VGCC Ab.

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

10. Modifications of Instructions for Use

Creation of instructions for use. No changes to declare.

11. Literature

- V.A. Lennon, Th.J. Kryzer, G.E. Griesmann, P.E. O'Suilleabhain, A.J. Windebank, A. Woppmann, G.P. Miljanich, E.H. Lambert
  Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes.
  N. Engl. J. Med. 1995;**332**:1467-1474

- M. Motomura, I. Johnston, B. Lang, A. Vincent, J. Newsom-Davis
  An improved diagnostic assay for Lambert-Eaton myasthenic syndrome.
  J. Neurol. Neurosurg. Psychiatry 1995;**58**:85-87

Pipetting Scheme

Allow all reagents, except Assay Buffer to stand at room temperature (20-25 °C) for at least 30 minutes before use.

		Negative Control	Positive Controls	Samples
[CON -]	µl	50		
[CON + I] & [CON + II]	µl		50	
Samples (Diluted 1:10 in Assay Buffer)	µl			50
[TRACER]	µl	50	50	50

Vortex gently, in 2 tubes determine total counts for 1 minute
Cover and incubate for 16-20 hours at 2-8 °C

[ANTI-HUMAN-IGG]	µl	50	50	50

Vortex gently, cover and incubate for 1.5 hours at 2-8 °C

[BUFFER] (cold)	ml	1	1	1

Centrifuge for 20 minutes at 1500 x g at 2-8 °C
Aspirate or decant supernatant carefully

[BUFFER] (cold)	ml	1	1	1

Vortex gently to resuspend pellet
Centrifuge for 20 minutes at 1500 x g at 2-8 °C
Aspirate or decant supernatant carefully
Determine counts in tubes for 1 minute in a gamma counter