RiaRSR™ Canine AChR Ab

Canine Acetylcholine Receptor Autoantibody RIA Kit - Instructions for use

FOR RESEARCH USE ONLY

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INTENDED USE

The RSR Canine Acetylcholine Receptor autoantibody (cAChR Ab) RIA kit is intended for use by professional persons only, for the quantitative determination of cAChR Ab in canine serum. Canine serum autoantibodies reactive with canine acetylcholine receptor (cAChR) are implicated in impaired neuromuscular transmission at the neuromuscular junction, specifically associated with canine myasthenia gravis (MG). Measurement of the antibodies can be of considerable value in disease diagnosis.

REFERENCES

C.W Dewey et al
Clinical Forms of Acquired Myasthenia Gravis in Dogs: 25 Cases (1988-1995).
J. of Veterinary Internal Medicine (1997) 11: 50 – 57

G.D Shelton et al
Acquired Myasthenia Gravis. Selective Involvement of Esophageal, Pharyngeal and Facial Muscles.
J. of Veterinary Internal Medicine (1990) 4: 281 – 284

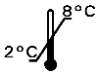
ASSAY PRINCIPLE

The assay depends on the use of recombinant canine AChR complexed with ^{125}I-labelled alpha bungarotoxin. The ^{125}I-labelled cAChR are then incubated with test sera and the resulting complexes immunoprecipitated with anti-IgG antibody. 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. 10 µL is sufficient for one assay (duplicate 5 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 10,000 g 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

3.5 mL assay tubes (round bottomed tubes are recommended when using precipitation enhancer)
Suitable rack for assay tubes
Pipettes capable of dispensing 5 µL, 25 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 Canine AChR ~ 20kBq/vial** 2 vials (at manufacture) Lyophilised
	Reconstitute each vial with 0.75 mL of reconstitution buffer (B) and mix gently to dissolve. **Use immediately.**
B	**Reconstitution Buffer for ^{125}I Labelled Canine AChR** 4 mL Ready for use
C	**Negative Control** 0.1 mL Ready for use

D	**Positive Control** (See label for concentration range) 0.1 mL Ready for use.
E	**Normal Serum** 1 mL Ready for use
F	**Anti-IgG Ab** 1.5 mL Ready for use
G	**Precipitation Enhancer** 1 mL Ready for use
	Mix thoroughly immediately before use
H	**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, 7 and 10.

1.	Pipette **5 µL** (in duplicate) of negative control (C), positive control (D) and test sera (undiluted), into labelled assay tubes.
2.	Pipette 50 µL of **freshly reconstituted** ^{125}I labelled cAChR (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-IgG Ab (F) 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 25 µL of precipitation enhancer (G) into each tube (excluding the two total count tubes).
7.	Pipette 1 mL of **cold** (2 – 8°C) wash solution (H) into each tube (excluding the two total count tubes) and mix gently on a vortex mixer.
8.	Centrifuge each tube at 1500g for 20 minutes at 2 – 8°C.
9.	Aspirate or decant the supernatants.
10.	Pipette 1 mL of **cold** (2 – 8°C) wash solution (H) into each tube (excluding the two total count tubes) and resuspend the pellet gently using a vortex mixer.
11.	Repeat steps 8 and 9.
12.	Count each tube (including total count tubes) for ^{125}I for 2 minutes using a gamma counter.

RESULT ANALYSIS

The radioactivity in the pellet represents the amount of ^{125}I-labelled cAChR bound by the cAChR Ab. This can be expressed as nanomoles of labelled cAChR bound per litre of test serum using the following equation:

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

where; **A** is the decay factor for ^{125}I between the receptor manufacture day and the day of assay; **B** is the counter efficiency; **C** is the volume of serum used in the assay (i.e. 5 µL) and **K** is the specific activity (Ci/mmol) of the ^{125}I-labelled cAChR, Values for A and K are provided with each kit lot on a separate sheet.

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

	cpm	nmol/L
Negative Control	1004	0.0
Positive Control	7688	4.1

ASSAY CUT OFF

Negative	< 1.0 nmol/L
Positive	≥ 1.0 nmol/L

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

Assay Linearity

The relationship between canine 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 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.

CLINICAL EVALUATION

Clinical Specificity

Sera from 24 individual healthy dogs were assayed in the cAChR Ab RIA. 23 (96%) were identified as being negative for cAChR Ab.

Clinical Sensitivity

Sera from 4 dogs diagnosed with myasthenia gravis were assayed in the cAChR Ab RIA. All 4 were identified as being positive for cAChR Ab.

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

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. 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 after handling. Monitor hands and clothing before leaving the designated area. 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, **(excluding wash solution (H))** and samples to stand at room temperature (20-25°C) for at least 30 minutes before use	
Pipette:	**5** µL negative control (C), positive controls (D) and test sera (all undiluted)
Pipette:	**50** µL ^{125}I labelled cAChR (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
Pipette:	**50** µL anti-IgG Ab (F) into all tubes (excluding the two total count tubes)
Mix:	Mix tubes gently on vortex mixer and cover
Incubate:	2 hours at room temperature
Pipette:	**25** µL precipitation enhancer (G) into all tubes (excluding the two total count tubes)
Pipette:	**1 mL cold** (2 – 8°C) wash solution (H) (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
Pipette:	**1 mL cold** (2 – 8°C) wash solution (H) (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
Count tubes for ^{125}I for 2 minutes using a gamma counter	