RSR

RiaRSR™ MuSK Ab

Muscle Specific Tyrosine Kinase (MuSK) Autoantibody RIA Kit - Instructions for use

CE

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INTENDED USE

The RSR Muscle Specific Tyrosine Kinase (MuSK) autoantibody (Ab) RIA kit is intended for use by professional persons only, for the quantitative determination of MuSK autoantibodies in human serum. Autoantibodies to the MuSK protein have been found to lead to the failure of neuromuscular transmission and muscle weakness associated with acetylcholine receptor autoantibodies (AChRAb) seronegative myasthenia gravis (MG). MuSK is a skeletal muscle-specific protein that is essential for neuromuscular junction formation. Measurement of these antibodies can be of considerable value in disease diagnosis and management.

REFERENCES

W. Hoch et al
"Auto-Antibodies to the receptor tyrosine kinase MuSK in patients with Myasthenia Gravis without acetylcholine receptor antibodies."
Nat. Med. 2001 **7** :365 – 368

I. Matthews et al
"Muscle-specific receptor tyrosine kinase autoantibodies – a new immunoprecipitation assay"
Clinica Chimica Acta 2004 **348**: 95 – 99

ASSAY PRINCIPLE

In RSR's MuSK Ab radioimmunoassay (RIA), MuSK Ab in patient sera and controls are allowed to interact with ^{125}I-labelled MuSK protein (^{125}I-MuSK). After incubation at room temperature overnight, the resulting antigen-antibody complexes are immunoprecipitated by the addition of anti-human IgG. After a second incubation of 2 hours, precipitation enhancer and wash solution are added and the samples centrifuged. Unbound ^{125}I-MuSK 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 or below –20°C. 15 µL is sufficient for one assay (duplicate determinations). Repeated freeze thawing or increases in storage temperature must be avoided. Do not use lipaemic or haemolysed serum samples. Do not use plasma in the assay. When required, bring test sera to room temperature and mix gently to ensure homogeneity. Centrifuge 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. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

SYMBOLS

Symbol	Meaning
CE	EC Declaration of Conformity
IVD	In Vitro Diagnostic Device
REF	Catalogue Number
LOT	Lot Number
	Consult Instructions
	Manufactured by
Σ	Sufficient for
	Expiry Date
2°C 8°C	Store
CONTROL -	Negative Control
CONTROL +	Positive Control

MATERIALS REQUIRED AND NOT SUPPLIED

4.5 mL Conical plastic tubes
Pipettes capable of dispensing 25 µL, 50 µL, 1 mL and 1.5 mL
Vortex mixer
Refrigerated centrifuge capable of 1500g
Gamma counter

PREPARATION OF REAGENTS SUPPLIED

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

A	**Negative Control** 0.25 mL Diluted 1:10 Ready for use
B	**Positive Control** 0.25 mL Diluted 1:10 Ready for use.

C	**^{125}I-Labelled MuSK** ~30kBq/vial 1 vial (at manufacture) Lyophilised
	Reconstitute the vial by addition of 1.5 mL Reconstitution Buffer (D) and vortex gently to dissolve. **Use immediately.**
D	**Reconstitution Buffer** 4 mL Ready for use
E	**Anti-Human IgG** 1.5 mL Ready for use (Variations in appearance may occur without influence on assay performance).
F	**Precipitation Enhancer** 1 mL Ready for use
G	**Wash Solution** 70 mL Ready for use

ASSAY PROCEDURE

Allow all reagents, 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 50 µL of test sera diluted 1:10 in wash solution (G), 50 µL of negative control (A) or 50 µL of positive control (B) into labelled 4.5 mL conical plastic tubes (in duplicate is recommended). The negative and positive controls are supplied ready diluted so do not dilute again and always use 50 µL per tube. Alternatively, 5 µL of undiluted test sera may be used.
2.	Pipette 50 µL of **freshly reconstituted** ^{125}I-labelled MuSK (C) 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 16 - 20 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 2 – 8°C for 2 hours.
6.	Pipette 25 µL of precipitation enhancer (F) into each tube (excluding the two total count tubes).
7.	Pipette 1 mL of wash solution (G) 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 4°C.
9.	Aspirate or decant the supernatant.
10.	Pipette 1 mL of wash solution (G) 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 1 minute using a gamma counter.

RESULT ANALYSIS

The radioactivity in the final pellet is proportional to the amount of labelled MuSK bound by MuSK Ab. This can be expressed as nmol of labelled MuSK bound per litre of test serum using the following equation:

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

where;

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

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

	cpm	nmol/L
Negative Control	249	0
Positive Control	10103	0.746

ASSAY CUT OFF

Negative	<0.05 nmol/L
Positive	≥0.05 nmol/L

This cut off has been validated at RSR. However, each laboratory should establish its own normal and pathological reference ranges for MuSK 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 MuSK Ab RIA. 50 (100%) were identified as being negative for MuSK Ab.

Clinical Sensitivity

Serum samples from 18 patients with clinical symptoms of MG but negative for AChR Ab were assayed in the MuSK Ab RIA. 18 (100%) were positive for MuSK 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 0.0023 nmol/L.

Intra Assay Precision

Sample	Mean nmol/L (n=25)	CV (%)
1	1.2	3.8
2	0.66	5.4
3	0.06	7.2

Inter Assay Precision

Sample	Mean nmol/L (n = 20)	CV (%)
A	0.79	8.4
B	0.48	8.7
C	0.39	5.3
D	0.11	7.8
E	0.04	12.2

Clinical Accuracy

Analysis of 13 patients with autoimmune diseases other than MG indicated no interference from autoantibodies to aquaporin-4 (n = 3), 21-hydroxylase (n = 5) and glutamic acid decarboxylase (n = 5).

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.

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 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 Sheets for more detailed safety information. The kit contains radioactive material ^{125}I (half-life: 60 days), emitting ionizing x-ray (28 keV) and gamma (35.5 keV) radiations. 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 to stand at room temperature (20-25°C) for at least 30 minutes before use	
Pipette:	50 µL of patient sera diluted 1:10 in wash solution (G), 50 µL of negative (A) and positive control (B) (the controls are supplied ready diluted do not dilute again and always use 50 µL per tube). Alternatively 5 µL of undiluted test sera may be used
Pipette:	50 µL ^{125}I-labelled MuSK (**freshly reconstituted** (C)) into all tubes plus two additional empty tubes for total counts
Tubes:	Mix gently on vortex mixer and cover
Incubate:	16 - 20 hours at room temperature (20-25°C)
Pipette:	50 µL Anti-human IgG (E) into all tubes (excluding the two total count tubes)
Tubes:	Mix gently on vortex mixer and cover
Incubate:	2 hours at 2-8°C
Pipette:	25 µL precipitation enhancer ((F) excluding the two total count tubes)
Pipette:	1 mL wash solution ((G) 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 wash solution (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	