DIAGNOSTIKA GMBH

Instructions for Use

MuSK Antibody RIA

^{125}I-Radioimmunoassay for the
Quantitative Determination of
MuSK Autoantibodies in Serum

CE IVD

REF RA120/25

Σ 25

+8 +2 °C 2 – 8 °C

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Table of Contents

Symbols

IVD	In Vitro Diagnostic Medical Device	CE	EC Declaration of conformity
CONT	Content		Expiry Date
LOT	Lot Number		Store at
EC REP	EU Representative	Σ	Sufficient for ... determinations
REF	Catalogue Number		Consult Instructions for Use

Hazard Pictograms

Radioactive

Warning

1 Introduction and Principle of the Test

The Muscle Specific Tyrosine Kinase (MuSK) Antibody 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.

In the MuSK Antibody RIA, MuSK antibodies in patient sera 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.

2 Precautions

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. 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.

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

3 Storage and Stability

Upon 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

^{125}I-labelled MuSK 1 vial

Activity < 30 kBq, lyophilized

Radioactive

Negative Control **CONTROL -** 1 vial

0.25 ml Serum, ready for use

Positive Control **CONTROL +** 1 vial

0.25 ml Serum, ready for use, human serum containing antibodies against MuSK, for concentration ranges see qc certificate

Anti Human IgG 1 vial

1.5 ml, ready for use

Precipitation Enhancer 1 vial

1 ml, ready for use

Warning

Reconstitution Buffer 1 vial

4 ml, ready for use, for reconstitution of tracer

Wash Solution 1 vial

70 ml, ready for use

Additional materials and equipment required but not provided:

- Variable pipettes for dispensing 5 µl up to 1.5 ml
- 4.5 ml conical plastic tubes and suitable rack
- Refrigerated centrifuge capable of 1,500 x g.
- Vortex mixer
- Gamma Counter

5 Specimen Collection and Storage

Sera can be stored up to one week at 2 – 8 °C or at -20 °C for longer periods, preferably in aliquots. 15 µL is sufficient for one assay with 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.

6 Preparation of Samples and Reagents

6.1 Samples

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

Centrifuge sera prior to assay (preferably for 5 min at 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.

6.2 Dilution of Samples

Prior to assay, dilute sera 1:10 with Wash Solution (e.g. 15 µl serum plus 135 µl Wash Solution). 50 µl of the 1:10 diluted serum are needed per tube. Alternatively, 5 µl of undiluted serum per tube can be used in the assay.

6.3 Reconstitution of Tracer

Approximately 30 minutes before use, reconstitute the ^{125}I-labelled MuSK tracer with 1.5 ml Reconstitution Buffer and vortex gently to dissolve. The reconstituted tracer contains about 50,000 cpm per 50 µl and can be used for one week after reconstitution. Store at 2-8 °C.

All other components are ready for use.

7 Test Procedure

1. Pipette 50 µl Negative Control, 50 µl Positive Control and 50 µl of 1:10 diluted sera (alternatively, 5 µl neat sera may be used), each in duplicate, into labelled conical plastic tubes.
2. Add 50 µl ^{125}I-MuSK to each tube and mix gently on a vortex mixer.
3. Determine the total radioactivity (cpm) in three tubes for 1 or 2 minutes using a gamma counter.
4. Cover the tubes with a suitable cover and incubate over night (16-20 hours) at room temperature (20-25 °C).
5. Add 50 µl Anti-human IgG to each tube and gently mix on a vortex mixer.
6. Cover the tubes with a suitable cover and incubate for 2 hours at 2-8 °C.
7. Mix the Precipitation Enhancer thoroughly on a vortex mixer and add 25 µl to each tube.
8. Add 1 ml Wash Solution to each tube.
9. Centrifuge all tubes for 20 minutes at 1,500 x g (option: 3,000 x g) at 4 °C.
10. Decant or aspirate the supernatant, taking care not to disturb the pellet.
11. Add 1 ml of Wash Solution to each tube and gently resuspend the pellets using a vortex mixer for at least 10 seconds.
12. Centrifuge all tubes for 20 minutes at 1,500 x g (option: 3,000 x g) at 4 °C.
13. Decant or aspirate the supernatant, taking care not to disturb the pellet.
14. Determine the cpm in each tube for 1 or 2 minutes using a gamma counter.

8 Calculation of Results

The radioactivity in the final pellet is proportional to the amount of ^{125}I-labelled MuSK bound by MuSK Antibodies. This can be expressed as nmol of labelled MuSK bound per liter of test serum using the following equation:

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

With:

- A = specific activity [Ci/mmol] of the ^{125}I-labelled MuSK at the time it was labelled (see qc certificate included in each kit).
- B = the counter efficiency of the gamma counter used (e.g. 70%, then B = 0.7)
- C = volume of serum used in the assay (5 µl, see Dilution of Samples).
- D = decay factor for ^{125}I between the MuSK labelling day and the day of the assay (see qc certificate included in each kit).

The denominator can be calculated separately as the factor F:

$$F = \frac{1}{A \times B \times C \times 2.22}$$

The qc certificate included in each kit states an F value specific for the lot. Should the counter efficiency C of your gamma counter differ from the one stated on the qc certificate, then F has to be recalculated accordingly.

Therefore the above formula becomes simplified to:

$$\text{MuSK-Ab [nmol/l]} = (\text{cpm}_{\text{Pos. Control OR sample}} - \text{cpm}_{\text{Neg. Control}}) \times D \times F$$

For assay to be valid, the value achieved for the positive control must be in the target range indicated on qc certificate included in each kit.

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

	cpm	**cpm - cpm$_{\text{Neg. Control}}$**	**MuSK-Ab [nmol/l]**
Negative Control	249	0	0
Positive Control	10,103	9,854	0.746

9 Assay Characteristics

9.1 Assay Cut Off

Negative	< 0.05 nmol/l
Positive	≥ 0.05 nmol/l

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

9.2 Clinical Evaluation

9.2.1 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.

9.2.2 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.

9.2.3 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.

9.2.4 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

9.2.5 Inter Assay Precision

Sample	Mean nmol/l (n=20)	CV [%]
A	0.79	4.8
B	0.48	8.7
C	0.39	5.3
D	0.11	7.8
E	0.04	12.2

9.2.6 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).

9.2.7 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.

10 Literature

- 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

- Matthews I., Chen S., Hewer R., McGrath V., Furmaniak J., Rees Smith B.
  Muscle-specific receptor tyrosine kinase autoantibodies – a new immunoprecipitation assay
  Clinica Chimica Acta 2004 348: 95 – 99

11 Changes to declare

Section 5: “Sera should be assayed soon after separation” was replaced with “Sera can be stored up to one week at 2 – 8 °C”.

Section 7 and Pipetting Scheme: In steps 3 and 14 “or 2 minutes” was added. In steps 9 and 13 “(option: 3,000 x g)” was added.

Pipetting Scheme

Allow reagents to equilibrate to room temperature (20-25 °C) for at least 30 minutes

		Negative Control	Positive Control	Sample
Negative Control	µl	50		
Positive Control	µl		50	
1:10 dil. serum	µl			50
^{125}I-labelled MuSK	µl	50	50	50

Mix gently on vortex mixer
Determine cpm in 3 tubes for 1 or 2 minutes using gamma counter
Cover tubes with suitable cover
Incubate over night at room temperature

Anti Human IgG	µl	50	50	50

Mix gently on vortex mixer
Cover tubes with suitable cover
Incubate for 2 hours at 2-8 °C

Precipitation Enhancer	µl	25	25	25
Wash Solution	ml	1	1	1

Mix gently on vortex mixer
Centrifuge at 1,500 x g (option: 3,000 x g) for 20 minutes at 4 °C
Aspirate or decant supernatant carefully

Wash Solution	ml	1	1	1

Gently resuspend pellet using vortex mixer
Centrifuge at 1,500 x g (option: 3,000 x g) for 20 minutes at 4 °C
Aspirate or decant supernatant carefully
Determine cpm in tubes for 1 or 2 minutes using a gamma counter