

## Anti-Striated Muscle Antibody Test System

### QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.
3. The positive serum controls should result in bright 3+ to 4+ fluorescence. If these controls show little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from non-specific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

### RESULTS

The IFA test will result in a cross-striation staining pattern of skeletal muscle. The percentages of patients with anti-striated antibodies vary with the clinical state:

Patient Population	% Anti-Str Abs
All MG patients	40%
MG with Thymoma	90-100%
MG without Thymoma	30%
Thymoma without MG	25%

\*Absence of anti-Str Abs effectively excludes thymoma.

### TEST LIMITATIONS

1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.
2. More than one mechanism may be involved in MG. Factors like sex, age, presence or absence of thymoma, other autoantibodies, HLA type, response to thymectomy and/or immunosuppressive drugs must be considered in addition to the detection of anti-AChR and anti-Str antibodies.

### LITERATURE REFERENCES

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**EC REP**

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### INSTRUCTIONS FOR USE

## Anti-Striated Muscle Antibody Test System

**K4819** - 48 Tests

**K9619** - 96 Tests

**K5019** - 50 Tests

**K0019** - 100 Tests

**K25019** - 250 Tests

Also for: **Monkey striated muscle slides**

**S4207** - 4 well

**S8207** - 8 well

**S5207** - 5 well

**S0207** - 10 well

**Rat striated muscle slides**

**S4107** - 4 well

**S8107** - 8 well

**S5107** - 5 well

**S0107** - 10 well

### INTENDED USE

The Bio-Diagnostics Anti-Striated Muscle Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of antibodies to striated muscle, in human serum.

### SUMMARY AND EXPLANATION

Clinical and experimental evidence strongly suggests that circulating antibodies directed against acetylcholine receptor (AChR) and muscle cell antigens are important in the pathogenesis of myasthenia gravis (1-3). Several antibody mediated mechanisms have been implicated in the alteration of neuromuscular transmission, including 1) complement mediated destruction of motor end plates with consequent AChR loss, 2) blockage of the AChR active site preventing AChR access, or 3) alteration of AChR turnover (4, 5). The positive response to thymectomy in patients with a short history of myasthenia gravis (MG) may be due to alteration of thymic cell populations that normally regulate antibody production (6, 7). Tests for detection of antibodies to AChR and muscle cell antigens can be of diagnostic value (8). High titres of anti-AChR and anti-striated (Str) muscle antibodies define MG patients with thymoma (9). The absence of anti-AChR or anti-Str antibodies effectively excludes MG or thymoma respectively (10). Because antibody titre to either AChR or Str muscle corresponds only approximately to clinical status, their detection does not have direct prognostic value (11-13). Radioimmunoassay is used for AChR antibody detection, whereas routine indirect immunofluorescence (IFA) is used for detection of anti-striated antibodies. Acetone fixed longitudinal sections of skeletal muscle is the substrate used for anti-striated antibody detection (14). Normal human sera can react with skeletal muscle in dilutions up to 1:30. A suggested screening dilution of 1:40 is recommended to increase specificity (10, 15).

### PRINCIPLE OF THE TEST

The primary reaction involves circulating anti-striated muscle antibodies present in the patient's serum. The binding of the antibody to its homologous antigen site occurs during the first incubation period whilst the serum covers the antigen surface. A secondary reaction then follows a rinsing period that removes the unbound human antibody. The reagent used in the secondary reaction is a fluorescein labelled antihuman globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope.



## Anti-Striated Muscle Antibody Test System

### WARNINGS AND PRECAUTIONS

1. The human components in the controls have been screened and found to be negative for HBsAg and antibodies to HIV-1, HIV-2, RPR and HCV. However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The controls included in the kit contain 0.1% sodium azide or 0.01% thiomersal as preservatives. Although these are at low concentrations, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
5. For in vitro diagnostic use.
6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artefacts.

### KIT CONTENTS

<b>SLIDE</b>	Monkey striated muscle substrate antigen slides (S4207, S5207, S8207 or S0207)
<b>CONJ IgG</b>	FITC Conjugate (for use with Primate substrates) with Evans Blue Counterstain: J502. This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
<b>CONTROL +</b>	Striated muscle Positive Control no: C015N/C015N-0.5
<b>CONTROL -</b>	Universal Negative Control no: C000N/C000N-0.5
<b>IFA/DFA PBS</b>	Buffer Pack no: R002
<b>MM</b>	Mounting Medium no: R005

Note: All kit components are available separately. Please see the Bio-Diagnostics Ltd catalogue for more details.

### ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes and rack or microtitre system	Disposable pipettes
Staining Dish and Slide Forceps	Moisture Chamber
Volumetric Flask (500 ml)	Distilled Water
Fluorescence Microscope	Paper Towels – lint free

### REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

### KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

	Manufacturer		Contains sufficient for <n> tests	<b>RFU</b>	Ready for use
	Temperature limitation	<b>IVD</b>	In vitro diagnostic medical device		

## Anti-Striated Muscle Antibody Test System

### STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents. Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

### SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

### TEST INSTRUCTIONS

**Screening:** dilute test serums 1/40 (1 part patient sample to 39 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.  
**Titration:** set up doubling dilutions of serum starting at 1/40, (i.e. 1/40, 1/80, 1/160, 1/320, etc.).

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluted serum (20 to 30µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 18-24°C).
4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Carefully dry the back and edges of the slide using a paper towel. **Do not allow tissue to dry.**
7. Deliver 1 drop (20-30µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope.

Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.