SZABO SCANDIC

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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

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For technical assistance please contact:

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: (716) 691-0091
Fax: (716) 691-0466
Toll Free USA/Canada: 1-800-537-TEST
E-Mail: info@immcodiagnostics.com

or your local product distributor

EC REP EU Authorized Representative/Autorisierter Repräsentant/Rappresentante Autorizzato/Representante Autorizado/Représentant Autorisé
EMERGO Group, Inc.
Molenstraat 15, 2513 BH, The Hague,
The Netherlands
Tel (+31) 345 8570, Fax (+31) 346 7299
www.emergogroup.com

ImmuGlo™
Autoantibody Test System I

For *in vitro* Diagnostic Use

PRODUCT INSERT

Cat. No. 1107-1

ImmuGlo™ Mouse Kidney Substrate, *Kit*
48 Determinations

Cat. No. 1107-2

ImmuGlo™ Mouse Kidney Substrate, *Reagent Package*
160 Determinations

INTENDED USE

For the detection and quantitative determination of antinuclear (ANA), antimitochondrial (AMA), and antismooth muscle (ASMA) antibodies in human serum by the indirect fluorescent antibody technique.

SUMMARY AND EXPLANATION

Immunofluorescence, or the fluorescent antibody technique, was developed by Coons in the early 1940s,[1] and defined quantitatively by Beutner and associates in the 1960s.[2] The use of fluorescein labeled antibody allows the precise localization of reaction sites in tissues. The ImmuGlo™ Autoantibody Test System I utilizes the indirect immunofluorescence methodology for detection of autoantibodies. This immunofluorescent staining is capable of detecting multiple autoantibodies; however, care in the interpretation of patterns is necessary. Elevated titers of autoantibodies are usually consistent with the diagnosis of an autoimmune disease. High titers of ANA are usually present in many of the collagen diseases.[3,4] In many cases, normal human sera may contain low levels of ANA as demonstrated by this technique.[4a] High titers of AMA are usually associated with primary biliary cirrhosis. Low levels of AMA, on the other hand, may also be detected in other diseases of the liver.[5-7] The highest incidence of ASMA is found in chronic active hepatitis (80%). A low percentage of the normal population has antibodies to smooth muscle.[8-10] ASMA incidence in viral hepatitis B does not differ significantly from the occurrence in the normal population.[11]

PRINCIPLES OF THE PROCEDURE

Sections of mouse kidney containing vessels with smooth muscle layers and mitochondria as well as nuclei are used as the antigen substrate. When this substrate is overlaid with the patient's serum, ANA, AMA and ASMA antibodies, if present, will bind to the specific antigen sites in the substrate.

The excess serum is then removed by washing and fluorescein-labeled antihuman IgG is added. After incubation, the slide is washed to remove unbound fluorescein-labeled IgG and observed under the fluorescence microscope.

The presence of ANA, AMA or ASMA in the serum sample is demonstrated by a yellowish-green (apple green) fluorescence when observed under a fluorescence microscope. Autoantibodies bound by the substrate will result in a predominant, observable pattern of fluorescence. The titer of autoantibodies is the reciprocal of the highest dilution of sample producing fluorescence.

REAGENTS

Composition

Anti-Human IgG Conjugate (H&L chain specific, Goat) fluorescein labeled (F/P ratio 1.5) in a buffer with 0.1% sodium azide, 6 or 16 mL
ANA Positive Control (Human) in buffer containing 0.1% sodium azide, 0.5 mL
Negative Control (Human) in buffer containing 0.1% sodium azide, 0.5 mL
Substrate Slides (Mouse Kidney), 8 wells/slide, with desiccant
Buffered Diluent, pH 7.1 (Buffer containing 0.1% sodium azide), 60 mL or 375 mL
Phosphate Buffered Saline Powder, pH 7.1 (Buffer containing sodium chloride), sufficient for 1000 mL
Mounting Medium (Buffered alcohol polymer solution), 0.07% sodium azide, 5 mL
The reagents contain preservatives and stabilizers

Warning

Sodium azide may react with copper or lead plumbing to form highly explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide buildup.

Precautions

For *in vitro* Diagnostic Use. Individual blood donations for preparation of the controls were examined for hepatitis B surface antigen and for antibodies to HIV by FDA required tests and only those found to be non-reactive were used.

SPECIFIC PERFORMANCE CHARACTERISTICS

The fluorescein labeled anti-human IgG conjugate provided with this kit was purposely chosen to provide proper sensitivity and specificity. Reports from the literature indicate that IgG is by far the predominant immunoglobulin class seen in SLE patients, with one study reporting 96 of 100 SLE patients positive for IgG class antinuclear antibody.[17] Another study indicated that low levels of antinuclear antibodies of IgM and IgA classes are seen in the normal population.[18]

The mouse kidney substrate provides antigens for the detection of antinuclear, antimitochondrial, and antismooth muscle antibodies.

The ImmuGlo™ Autoantibody System I is designed to detect ANA at titers of 20 or greater, and AMA and ASMA at titers of 10 or greater.

The accuracy of the ImmuGlo™ Autoantibody System I was established by comparison of results with alternative indirect immunofluorescence systems. In a series of 79 positive ANA specimens, 18 positive AMA specimens, and 10 positive ASMA specimens, comparison with other tissue section systems showed equivalent results.

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EXPECTED VALUES

The expected values for healthy adults using the ImmuGlo™ Autoantibody System I for ANA and AMA is the absence of a positive reaction at a 1:20 dilution of test specimen for ANA, and a 1:10 dilution for AMA. When a series of 162 sera were obtained from adult males and females and titered with the ImmuGlo™ Autoantibody System I, the following results were obtained.

Titer of ANA	Number of Sera
less than 20	160
greater than 20	2

None of the above 162 sera gave a positive response for AMA.

Another study of ANA levels in 96 normal blood donors, as well as patients with systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS) and rheumatoid arthritis (RA) yielded the following results:

Sera Tested		ANA Titers (Percent) Neg.	10-20	40-80	>160
Normal	(96)	97%	3%	-	-
SLE	(24)	4%	-	8%	88%
PSS	(17)	6%	12%	29%	53%
RA	(20)	75%	15%	10%	-

Approximately 20% of normal populations may show positive ASMA responses at a 1:10 dilution.[15, 16]

The results of this test may not correlate with systemic lupus erythematosus if the patient has been treated with corticosteroids or immuno-suppressive drugs.

WARNING - POTENTIAL BIOHAZARD

Since no test method can insure that infectious agents are absent, handle following good laboratory practices.[12]

Preparation

1. ***Anti-Human IgG Conjugate***
   Ready for use as supplied. Do not use if solution is turbid.
2. ***Phosphate Buffered Saline Powder***
   Transfer contents of one vial to a suitable vessel and completely dissolve by stirring in sufficient distilled water to make 1000 mL of solution. Label container and store at 2°C to 8°C.
3. ***Positive and Negative Controls***
   Ready to use as supplied. Do not use if solution is not clear.

Storage and Stability

Store at 2°C to 8°C. The phosphate buffered saline powder vials, cover slips, and buffered diluent may be stored at room temperature. Phosphate buffered saline solution is stable for 60 days at 2°C to 8°C.

Do not freeze conjugate. Freeze-thawing may reduce potency.

Specimens Collection and Preparation

This procedure should be performed with serum specimens only.

Interfering Substances

Turbidity, hemolysis, visible bacterial growth, or drugs capable of fluorescing in the test specimen may interfere with the performance and accuracy of the test. **Do not use turbid or lipemic serum specimens**.

Storage

Serum specimens may be stored at 2° to 8°C for up to 5 days. If longer storage is desired, store at -20°C.

PROCEDURE

Materials Provided

ImmuGlo™ Autoantibody Test System I Kit (***Cat. No. 1107-1)***

1 x 6 mL	Anti-Human IgG Conjugate
1 x 0.5 mL	ANA Positive Control *
1 x 0.5 mL	Negative Control *
6	Substrate Slides
1 x 60 mL	Buffered Diluent
2 vials	Phosphate Buffered Saline Powder
12	Cover Slips *
1 x 5 mL	Mounting Medium *

ImmuGlo™ Autoantibody Test System I, Reagent Package
(Cat. No. 1107-2)

1 x 16 mL	Anti-Human IgG Conjugate
20	Substrate Slides
1 x 375 mL	Buffered Diluent
3 vials	Phosphate Buffered Saline Powder

* ImmuGlo™ Autoantibody Test System I, Reagent Package, these materials are required but not provided.

Materials Required But Not Provided

Fluorescence microscope
Forceps, fine-tipped
Marking pencil
Moist chamber
Distilled or deionized water
Paper towels
Pasteur pipets and bulbs
Serologic pipets, 1.0 mL
Micropipets, 25 µL
Small test tubes
Containers, 1 liter
Coplin jar
200 mL beaker

Optional Materials

ImmuGlo™ AMA Positive Control (Antimitochondrial Antibody)	*Cat. No. 2210*
ImmuGlo™ ASMA Positive Control (Antismooth muscle Antibody)	*Cat. No. 2211*

Negative Reactions

The absence of specific fluorescent staining of the nucleus, nucleolus, mitochondria, or smooth muscle is considered negative for ANA, AMA, and ASMA. Unusual patterns which may be observed include the staining of the brush borders of the kidney tubules, the tubular basement membrane, the glomeruli (with an appearance of glomerular basement membrane staining), or of the connective tissue (with a pattern of reticulin antibodies). These patterns have not been found to have any clinical or diagnostic significance, but are sometimes mistaken for AMA antibodies. At least some of them are due to unusual heterophile antibodies.

All positive specimens should be titered (see “Titering of Positive Specimens”).

Determine the highest dilution producing a positive reaction. Both the titer and the pattern of the positive response should be determined.

LIMITATIONS OF THE PROCEDURE

The analyst should be aware of the possibility of antibody excess, nonspecific patterns and interference phenomena in reading slides.[13, 14]

Antibody excess may be observed when a significant excess of antibody occurs for antigen available, as in the case of high titers of ANA run at low dilutions. The result is a doubtful positive or negative reaction for a sample which yields a positive result at higher dilutions. In order to avoid false negatives, doubtful positives should be titered.

Interference phenomena may be due to the presence of two or more autoantibodies in a positive serum sample. When this occurs, one of the antibodies (usually the stronger one) predominates and suppresses the reaction of the other(s). Titering such sera may reveal first one and then another autoantibody. Repeat titerings often yield divergent titers. When this phenomenon occurs, the presence of all autoantibodies and their apparent titers should be reported. It should also be noted that the actual titer of the weaker autoantibody may have been suppressed.

Different clinical populations may warrant changing the initial screening for ASMA to dilutions higher than 1:10.

RESULTS

Reading of Slides

Examine several fields, reading those parts of the tissue sections with well defined cells for evaluation of ANA or AMA, or with blood vessels for evaluation of ASMA. Several fields of a single ring might have to be scanned in order to locate blood vessels required for the detection of ASMA. Initial examination at low magnification (100 - 200 x) may aid in locating the vessels. Not all cells will have optimal appearance due to possible disruption during sectioning. Identify any fluorescent staining observed on the sections as nuclear, nucleolar, mitochondrial or smooth muscle. Observed fluorescent staining should be compared to the appropriate positive and negative controls.

Positive Reactions for Antinuclear Antibodies (ANA)

Fluorescent staining of the nucleus with diffuse homogeneous, peripheral, speckled or nucleolar patterns is considered specific for antinuclear antibodies. Fluorescent staining of the nucleus may be classified by pattern as follows:

1. A **diffuse homogeneous** pattern is defined as a uniform, solid fluorescent stain throughout the nucleus.
2. A **peripheral** pattern has a characteristic fluorescent staining of the rim or edge of the nucleus.
3. A **nucleolar** pattern refers to fluorescent staining of the nucleolus.
4. A **speckled** pattern has numerous, discrete specks of fluorescent staining throughout the nucleus.

Positive Reaction for Antimitochondrial Antibodies (AMA)

Fluorescent staining of the mitochondria is considered specific for antimitochondrial antibodies.

Positive Reaction for Antismooth Muscle Antibodies (ASMA)

Fluorescent staining of the smooth muscle of blood vessels is considered specific for anti-smooth muscle antibodies. A positive sample should produce fluorescence on the inner and outer lining of the vessel.

Method

A. <u>Screening Test</u>

ANA Procedure - Dilute serum 1:20 with buffered diluent
ASMA and AMA Procedure - Dilute serum 1:10 with buffered diluent
DO NOT dilute Positive or Negative Controls.

1. Allow slide to warm to room temperature and then remove carefully from foil bag to avoid damaging the substrate.
2. Label slide on frosted end.
3. Place substrate slide in moist chamber.
4. For the antinuclear antibody (ANA) test, place ~25* µL of ANA Positive Control into ring #1 and ~25* µL of Negative Control into ring #2.
   For the antimitochondrial antibody (AMA) test, place ~25* µL of AMA Positive Control into ring #1 and ~25* µL of Negative Control into ring #2.
   For the antismooth muscle antibody (ASMA) test, place ~25* µL of ASMA Positive Control into ring #1 and ~25* µL of Negative Control into ring #2.
5. Place ~25* µL of properly diluted sample to each of the remaining rings.

* *Exact volumes are not critical, but it is important to avoid overflowing the rings. When using squeeze bottles, gently expel enough control to fill the ring. When using micropipets, carefully adjust flow to avoid spillage.*

6. Place lid on moist chamber and incubate slide for 30 minutes at room temperature. Remove slide from chamber and rinse with approximately 10 mL of phosphate buffered saline using a serologic pipet or wash bottle. Allow the solution stream to contact the end of the slide, preferably between the wells, not directly on the tissue sections. Wash gently but thoroughly. If the rinsing solution is applied directly or forcefully to the tissue section, streaking or tearing of the nuclei may occur.
7. Tap edge of slide on paper towel to remove excess phosphate buffered saline. Replace slide in moist chamber and immediately cover each well with antihuman IgG conjugate. DO NOT ALLOW SLIDE TO DRY. The conjugate dilution has been adjusted so that dilution by any phosphate buffered saline remaining on the slide will not interfere with the reaction.
8. Replace lid on moist chamber and incubate slide with antihuman IgG conjugate for 30 minutes at room temperature.
9. Remove slide from chamber, tap edge of slide on paper towel to remove conjugate, rinse with approximately 10 mL of phosphate buffered saline solution, then place into a Coplin jar containing fresh phosphate buffered saline solution for 10 minutes.

10. Remove slide from the Coplin jar and tap side of slide on paper towel to remove excess moisture. Apply cover slip using approximately 3 drops of mounting medium, taking care that air bubbles are expelled.
11. Examine slide under the fluorescence microscope. Patterns of fluorescence should be interpreted with caution in the screening test as the presence of multiple antibodies may influence the reading. Pattern interpretation is enhanced by the use of higher power immersion objectives to yield greater than 600X total magnification. Read slide within 60 minutes. If slide cannot be read within 60 minutes, it may be stored at 2° to 8°C in the dark for up to one month. Processed slides may be stored at -20°C for up to 6 months.

B. <u>Titering of Positive Specimens</u>

1. Prepare twofold serial dilutions of all specimens yielding a positive ANA, AMA or ASMA reaction in the screening test as follows:

ANA Serial Dilutions
Place 0.3 mL buffered diluent in each of five test tubes and add 0.3 mL of the initial 1:20 dilution to the first tube to provide a 1:40 dilution. With a clean serologic pipet, mix this 1:40 dilution thoroughly and transfer 0.3 mL of this mixture to the second tube to provide a 1:80 dilution. Repeat mixing and transfer steps, using a clean serologic pipet for each transfer, to provide three additional dilutions of the serum equivalent to 1:160, 1:320, and 1:640.

Dilution of ANA Positive Control
Squeeze one drop of Positive Control into 0.3 mL of buffered diluent. This will provide a weak positive control.

AMA or ASMA Serial Dilutions
Place 0.3 mL buffered diluent in each of five test tubes and add 0.3 mL of the initial 1:10 dilution of the first tube to provide a 1:20 dilution. With a clean serologic pipet, mix this 1:20 dilution thoroughly and transfer 0.3 mL of this mixture to the second tube to provide a 1:40 dilution. Repeat mixing and transfer steps, using a clean serologic pipet for each transfer, to provide three additional dilutions of the serum equivalent to 1:80, 1:160 and 1:320.

2. Repeat assay procedure given in steps 1 through 11 under “Screening Test” using the following specimens:

For ANA Titering
Use the dilution of the ANA Positive Control prepared as above, Negative Control, and the 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640 dilutions of the sample for titering.

For AMA and ASMA Titering
Use the AMA or ASMA Positive Control, the Negative Control, and the 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320 dilutions of the sample for titering.

3. Determine the highest dilution producing a positive reaction. If the highest dilution tested gives a positive reaction, further dilutions should be tested to measure the actual titer.

Quality Control

The positive control(s) should yield a positive reaction while the negative control should show no pattern of fluorescence. Occasional background fluorescence may be observed, and is usually due to improper washing techniques. The background fluorescence of the conjugate may be determined by running the screening test using the buffered diluent as the test sample. Little or no nonspecific background staining should be observed with the buffered diluent.

Control sera results outside of the expected range may be due to several factors:

a) improper storage of sera (look for signs of contamination, and if found, discard control sera).
b) improper use of optical system.
c) improper reading (compare carefully with negative control)
d) improper dilution techniques (repeat test following directions given under “Screening Test”)
e) partial drying of sera and/or conjugate (this can lead to false-negative reactions).

If a repeat test fails to yield the expected results, the controls and/or conjugate should be discarded and new reagents used.

If the samples spill into each other, discard the slide and repeat the test.