

DRG[®] Anti-Alpha-Fodrin IgG/IgA ELISA (EIA-3978)**Revised 29 July 2009 (Vers. 4.0)****RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

Anti-alpha Fodrin IgG/IgA is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG and IgA class autoantibodies against alpha-Fodrin in human serum or plasma.

The assay is intended for in vitro use only as an aid in the determination of Sjogren's syndrome (SS). In the United States, this kit is intended for Research Use Only.

SUMMARY AND EXPLANATION OF THE TEST

Alpha-Fodrin is an intracellular, actin-binding, organ-specific protein of the cytoskeleton. It is a dimer composed of an alpha- and a beta-subunit. The network of actin and fodrin situated below the plasma membrane of secretorial cells, is important for the alignment of secretorial vesicles to the plasma membrane during secretorial processes. During apoptosis the alpha-fodrin dimer is cleaved into a 120 kDa breakdown product, which is found abundantly in the salivary gland [2, 6]. This proteolysis of fodrin may be a consequence of protease activation during apoptosis [11]. The cleavage product of 120 kDa alpha-fodrin was found to be an important autoantigen in the pathogenesis of organ-specific autoimmune response [8]. Clinical studies have shown, that in patients with Sjögren Syndrome alpha-fodrin is involved in the stimulation of peripheral blood T-cells [2]. These findings suggest, that an increase in protease activity and the stimulation of T-cells play an important role in the alpha-fodrin proteolysis during the development of primary Sjogren's Syndrome [7].

The Sjogren's Syndrome is an autoimmune disorder affecting lachrymal and salivary glands (Sicca symptomatic). It is resulting in keratoconjunctivitis and xerostomia sicca. Sjogren's syndrome is elicited by lymphocytic infiltration of the lachrymal and salivary glands [9]. Sicca syndrome frequently affects patients with Grave's ophthalmopathy [13]. Alpha-fodrin autoantibodies are specifically detected in sera of adults affected with primary or secondary Sjogren's syndrome [2, 5, 12], but they are not found in other autoimmune disorders without sicca symptoms. In contrast to adults, the alpha-fodrin antibody is often present in juvenile SLE and juvenile RA without any signs suggesting secondary Sjogren's syndrome [10].

During an international congress in 1999 controversial data was presented regarding the prevalence of IgG and IgA antibodies directed against alpha-fodrin. A more recent study demonstrates a significantly higher prevalence of immunoglobulins class IgG [13]. In patients with SLE or Grave's ophthalmopathy and antibodies directed against alpha-fodrin, no correlation was found to antibodies directed against SS-A or SS-B [5, 13].

Kobayashi et al. detected anti-alpha-fodrin autoantibodies before anti-SS-A or anti-SS-B antibodies became positive [4]. Thus, the authors conclude, that anti-alpha-fodrin antibodies could be a useful marker for the early diagnosis of SS. The detection of anti-alpha-fodrin antibodies can prove to be a useful sensitive and specific marker for Sjogren's syndrome, particularly during the early stages of the pathogenesis of Sjogren's syndrome and even for diagnosis of juvenile SLE and RA. It has been shown that alpha-fodrin antibodies can be detected earlier than SS-A or SS-B. Clinical trials indicate that routine screening for alpha-fodrin antibodies is a valuable tool for the diagnosis of Sjogren's syndrome in adults with sicca symptoms and even for the determination of juvenile Sjogren's syndrome, SLE and RA. According to the latest findings, routine screening for antibodies directed against alpha-fodrin are a useful tool to diagnose Sjogren's syndrome in an early stage.

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PRINCIPLE OF THE TEST

Human alpha-Fodrin is bound to microwells. Antibodies to this antigen, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG or IgA immunologically bind to the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG resp. IgA antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro use only. In the United States, this kit is intended for Research Use Only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg and HIV by FDA approved methods. No test can guarantee the absence of HBsAg or HIV, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which contains hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations, though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8, 9., 10.)
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not Eat, Drink, Smoke or Apply Makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with human alpha-Fodrin. Ready to use.

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6 vials, 1.5 ml each	combined Calibrators with IgG and IgA class Anti-alpha-Fodrin antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN ₃ <0.1% (w/w)) containing: 0; 6.3; 12.5; 25; 50 and 100 U/ml. Ready to use.
2 vials, 1.5 ml each	Anti-alpha-Fodrin Controls in a serum/buffer matrix (PBS, BSA, NaN ₃ <0.1% (w/w)) positive for IgG (1), positive for IgA (2) and negative for IgG and IgA (3). Ready to use.
1 vial, 20 ml	Sample buffer (Tris, NaN ₃ <0.1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	Enzyme conjugate solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG ; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	Enzyme conjugate solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgA ; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution . Ready to use.
1 vial, 15 ml	Stop solution (1 M hydrochloric acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, NaN ₃ <0.1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8°C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8°C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- data reduction software

Preparation of reagents

- distilled or deionized water
- graduated cylinder for 100 and 1000 ml
- plastic container for storage of the wash solution

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1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28°C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

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PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples **1:100** with sample buffer before assay.

Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.

	1	2	3	4	5	6
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1/C2	P3			
F	SC	C1/C2	P3			
G	SD	C3	P4			
H	SD	C3	P4			

SA - SF: standards A to F

P1, P2... patient sample 1, 2 ...

C1/C2: positive controls for IgG or IgA

C3: negative control

3. Incubate for **30 minutes** at room temperature (20-28°C)
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well
6. Incubate for **15 minutes** at room temperature

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7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution
8. Dispense **100 µl** of TMB substrate solution into each well
9. Incubate for **15 minutes** at room temperature
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed color is stable for at least 30 minutes. Read optical densities during this time.

Automation

The Anti-alpha-Fodrin IgG/IgA ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS**Quality Control**

This test is only valid if the optical density at 450 nm for Positive Control (1 resp. 2) and Negative Control (3) as well as for the Standard A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not met, the results are invalid and the test should be repeated.

Calculation of results

For Anti-alpha-Fodrin IgG and IgA a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each Standard well. Use lin-log graph paper and plot the averaged optical density of each Standard versus the concentration. Draw the best fitting curve approximating the path of all Standard points. The Standard points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-alpha-Fodrin tests:

<u>Anti-alpha-Fodrin IgG/IgA</u>	
negative:	< 10 U/ml
positive	≥ 10 U/ml

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Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum anti-alpha-Fodrin.

PERFORMANCE CHARACTERISTICS**Parallelism**

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-alpha-Fodrin kit. The assay shows linearity over the full measuring range.

Precision (Reproducibility)

Statistics for Coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 6 different runs with 24 determinations each:

Intra-Assay		
Sample No	Mean [U/ml]	CV [%]
1	15.9	4.0
2	59.0	4.0
3	137.0	2.2

Inter-Assay		
Sample No	Mean [U/ml]	CV [%]
1	15.7	2.9
2	58.1	1.1
3	144.0	3.4

Sensitivity

The lower detection limit for Anti-alpha-Fodrin IgG/IgA was determined at 1.0 U/ml.

Specificity

The microplate is coated with human alpha-Fodrin. The Anti-alpha-Fodrin test kit recognizes only autoantibodies specific to the alpha-Fodrin.

Calibration

Since no international reference preparation for anti-alpha-Fodrin autoantibodies is available, the assay system is calibrated in relative arbitrary units.

LIMITATIONS OF PROCEDURE

The Anti-alpha-Fodrin IgG/IgA ELISA is a diagnostic aid and by itself is not diagnostic. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

A negative Anti-alpha-Fodrin result does not rule out the presence of Sjogren's syndrome

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INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera.

Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples be avoided.

REFERENCES

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..