ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49-51
55129 Mainz - Germany

Phone: +49 (0) 61 31 / 92 58-0
Fax: +49 (0) 61 31 / 92 58-58
Internet: www.orgentec.com

Instruction For Use
2013-10

ORG 642 Anti-alpha-Fodrin IgG/IgA

NAME AND INTENDED USE

Anti-alpha Fodrin IgG/IgA is an ELISA test system for the for the quantitative measurement of IgG and IgA class autoantibodies against alpha-Fodrin in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive IgG
CONTROL +	Control positive IgA
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE G	Enzyme Conjugate
CONJUGATE A	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Human alpha-Fodrin is bound to microwells.

Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect 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 colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

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 secretarial vesicles to the plasma membrane during secretorial processes. During apoptosis the alphafodrin 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 Sjögren's Syndrome [7].

The Sjögren's Syndrome is an autoimmune disorder affecting lachrymal and salivary glands (Sicca symptomatic). It is resulting in keratoconjunctivitis and xerostomia sicca. Sjögren'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 Sjögren´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 Sjögren´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 Sjögren´s syndrome, particularly during the early stages of the pathogenesis of Sjögren'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 Sjögren´s syndrome in adults with sicca symptoms and even for the determination of juvenile Sjögren ´s syndrome, SLE and RA. According to the latest findings, routine screening for antibodies directed against alpha-fodrin are a useful tool to diagnose Sjögren's syndrome in an early stage.

CONTENTS OF THE KIT

ORG 642	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***FOD***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 6.3 U/ml, containing alpha-fodrin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 12.5 U/ml, containing alpha-fodrin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 25 U/ml, containing alpha-fodrin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 50 U/ml, containing alpha-fodrin antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 100 U/ml, containing alpha-fodrin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive IgG, containing alpha-fodrin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL +	1x 1.5 ml	Control positive IgA, containing alpha-fodrin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing alpha-fodrin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE G	15 ml	Enzyme Conjugate; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
CONJUGATE A	15 ml	Enzyme Conjugate; containing anti-human IgA antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Instruction for Use: ELISA Mini-DVD
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.

- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production.
  Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C.
  We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	A	P1								
B	B	P2	B	P2								
C	C	P3	C	P3								
D	D	P4	D	P4								
E	E	P5	E	P5								
F	F	P6	F	P6								
G	C+	P7	C+	P7								
H	C-	P8	C-	P8								
	IgG	IgG	IgA	IgA								

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

CALIBRATION

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is IgG: 0 - 100 U/ml IgA: 0 - 100 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off IgG: 10 U/ml IgA: 10 U/ml

Interpretation of results

	IgG	IgA
Negative:	< 10 U/ml	< 10 U/ml
Positive:	≥ 10 U/ml	≥ 10 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
IgG 1	1:100	72.2	72.2	100
.	1:200	37.5	36.1	104
.	1:400	18.6	18.1	103
.	1:800	8.9	9.0	99
IgG 2	1:100	93.6	93.6	100
.	1:200	47.8	46.8	102
.	1:400	22.9	23.4	98
.	1:800	11.9	11.7	102
IgG 3	1:100	58.3	58.3	100
.	1:200	28.9	29.2	99
.	1:400	14.9	14.6	102
.	1:800	7.0	7.3	96
IgA 1	1:100	74.2	74.2	100
.	1:200	38.2	37.1	103
.	1:400	18.9	18.6	102
.	1:800	9.2	9.3	99
IgA 2	1:100	52.3	52.3	100
.	1:200	26.9	26.2	103
.	1:400	13.0	13.1	99
.	1:800	6.6	6.5	102
IgA 3	1:100	35.2	35.2	100
.	1:200	17.5	17.6	99
.	1:400	8.6	8.8	98
.	1:800	4.5	4.4	102

Limit of detection

Functional sensitivity was determined to be: IgG: 1 U/ml IgA: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay IgG		
Sample	Mean	.
.	U/ml	CV %
1	16.3	0.7
2	33.2	5.4
3	73.2	3.8

Inter-Assay IgG		
Sample	Mean	.
.	U/ml	CV %
1	16.6	3.6
2	33.1	7.1
3	75.6	4.9

Intra-Assay IgA		
Sample	Mean	.
.	U/ml	CV [%]
1	12.4	1.1
2	25.5	3.7
3	72.5	3.7

Inter-Assay IgA		
Sample	Mean	.
.	U/ml	CV [%]
1	12.0	4.7
2	25.1	5.3
3	70.6	4.3

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	Pos IgG	%	Pos IgA	%
Sjögren Syndrome	65	59	90.8	55	84.6
Rheumatoid Arthritis	70	23	32.9	23	32.9
Normal human sera	100	7	7.0	6	6.0

		Clinical Diagnosis		
		Pos	Neg	
ORG 642 IgG	Pos	82	7	
	Neg	53	93	
	.	135	100	235
Sensitivity:	60.7 %			
Specificity:	93.0 %			
Overall agreement:	74.5 %			

		Clinical Diagnosis		
		Pos	Neg	
ORG 642 IgA	Pos	78	6	
	Neg	57	94	
	.	135	100	235
Sensitivity:	57.8 %			
Specificity:	94.0 %			
Overall agreement:	73.2 %			

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. 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 concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Bell M, Askari A, Bookman A, Frydrych S, Lamont J, McComb J, Muscoplat C, Slomovic A. Sjögren´s syndrome: A critical review of clinical management. J Rheumatol 1999; 26:2051- 61.
2. Haneji N, Nakamura T, Takio K, Yanagi K, Higashiyama H, Saito I, Noji S, Sugino H, Hayashi Y. Identification of alpha-fodrin as a candidate autoantigen in primary Sjögren´s syndrome. Science 1997; 276 :604-7
3. Harley JB,Alexander EL, Bias WB, Fox OF, Provost TT, Reichlin M,Yamagata H,Arnett FC. Anti-Ro (SS-A) and anti-La (SS-B) in patients with Sjögren´s syn-drome. Arthritis Rheum 1986; 29:196-206.
4. Kobayashi I, Kawamura N, Okano M, Shikano T, Mizumoto M, Hayashi Y, Kobayashi K. Anti-alpha-Fodrin autoantibody is an early diagnostic marker for childhood primary Sjögren´s syndrome. J Rheumatol 2001; 28:363 -5.
5. Maeno N,Takei S, Imanaka H,Oda H,Yanagi K, Hayashi Y, Miyata K. Anti-alpha-Fodrin antibodies in Sjögren´s syndrome in children. J Rheumatol 2001; 28:860-4.
6. Martin SJ, O´Brien GA, Nishioka WN,McGahon AJ,Mahboubi A, Saido TC, Green DR. Proteolysis of fodrin (non-erythroid spectrin) during apoptosis. J Biol Chem 1995; 270:6425-8.
7. Saegusa K, Ishimaru N, Yanagi K, Haneji N, Nishino M, Azuma M, Saito I, Hayashi Y. Autoantigen-specific CD4 +CD28low T cell subset prevents autoimmune exocrinopathy in murine Sjögren´s syndrome. J Immunol 2000; 165:2251-2257.
8. Saegusa K, Ishimaru N, Yanagi K, Arakaki R, Ogawa K, Saito I, Katunuma N, Hayashi Y. Cathepsin S inhibitor prevents autoantigen presentation and autoimmunity. J Clin Invest 2002 Aug;110(3): 361-9
9. Sjögren H, Bloch K. Keratoconjuntivitis sicca and the Sjögren´s syndrome. Surv Opthalmol 1971; 16:145-59.
10. Takahashi K, Tatsuzawa O, Yanagi K, Hayashi Y, Takahashi H. Alpha-fodrin auto-antibody in Sjögren syndrome and other auto-immune diseases in childhood. Eur J Pediatr 2001; 160(8):520-1.
11. Vanags DM, Pörn-Ares MI, Coppola S, Burgess DH, Orrenius S. Protease involvement in fodrin cleavage and phosphatidylserine exposure in apoptosis. J Biol Chem 1996; 271:31075-85
12. Watanabe T, Tsuchida T, Kanda N, Mori K, Hayashi Y, Tamaki K. Anti-alpha-fodrin antibodies in Sjögren´s syndrome and lupus erythematosus. Arch Dermatol 1999; 135:535-9.