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Instruction For Use
2014-01



US Market: For Research Use Only

ORG 642 Anti-alpha-Fodrin IgG/IgA

NAME AND INTENDED USE

Anti-alpha Fodrin IgG/IgA is an ELISA test system 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

| | |
|--|--|
| | In vitro diagnostic medical device |
| | Manufacturer |
| | Catalogue number |
| | Sufficient for 96 determinations |
| | Batch code |
| | Use by |
| | Temperature limitation |
| | Consult instructions for use |
| | Keep away from sunlight |
| | Do not reuse |
| | Date of manufacture |
| | conform to European directive 98/79/EC |

| | |
|--|----------------------|
| | Microplate |
| | Calibrator |
| | Calibrator |
| | Calibrator |
| | Calibrator |
| | Calibrator |
| | Calibrator |
| | Control positive IgG |
| | Control positive IgA |
| | Control negative |
| | Sample Buffer P |
| | Enzyme Conjugate |
| | Enzyme Conjugate |
| | TMB Substrate |
| | Stop solution |
| | Wash Buffer |
| | Ready to use |

PRINCIPLE OF THE TEST

Human alpha-Fodrin is bound to microwells.

The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

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 | ▽ 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. |
| WASH | 20 ml | Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc. |
| STOP | 15 ml | Stop solution; contains acid. Ready to use. |
| i | 1 | Instruction for Use: ELISA Mini-DVD |
| i | 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 desiccated in the clip bag provided.
- Shelf life of the unopened 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, perform 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, classification 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.

- 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.
- Dispense **100 µl** 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.
- Dispense **100 µl** of TMB substrate solution into each well.
Incubate for **15 minutes** at room temperature
- 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

Negative: IgG < 10 U/ml IgA < 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 | 235 |
| | Neg | 53 | 93 | |
| | | 135 | 100 | |

Sensitivity: 60.7 %
Specificity: 93.0 %
Overall agreement: 74.5 %

| | | Clinical Diagnosis | | |
|-------------|-----|--------------------|-----|-----|
| | | Pos | Neg | |
| ORG 642 IgA | Pos | 78 | 6 | 235 |
| | Neg | 57 | 94 | |
| | | 135 | 100 | |

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 establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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- ① **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
→ **30 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschlösung waschen
- ② **100 µl** Enzymkonjugatlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschlösung waschen
- ③ **100 µl** Substratlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
- ④ **100 µl** Stopplösung zugeben
→ Platte **5 Minuten** stehenlassen
→ Bei **450 nm** messen



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