Alpha Fodrin IgG ELISA

For the quantitative and qualitative detection of IgG antibodies against α-Fodrin in human serum.

For Research Use Only. Not for Use in Diagnostic Procedures

Catalog Number: 35-AFGHU-E01
Size: 96 wells
Version: 003: 2015-09-24 - ALPCO 1.0

1. Intended Use

The Alpha Fodrin IgG ELISA is a solid phase enzyme immunoassay with recombinant human α-Fodrin for the quantitative and qualitative detection of IgG antibodies against α-Fodrin in human serum. For research use only. Not for use in diagnostic procedures.

2. Principle of the Assay

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Antibodies, if present in the sample, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.

3. Materials Provided

TO BE RECONSTITUTED				
Item	**Quantity**	**Cap color**	**Solution color**	**Description / Contents**
Sample Buffer (5x)	1 x 20mL	White	Yellow	5x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide <0.1% (preservative)
Wash Buffer (50x)	1 X 20mL	White	Green	50x concentrated Tris, NaCl, Tween, sodium azide <0.1% (preservative)

READY TO USE				
Item	**Quantity**	**Cap color**	**Solution color**	**Description / Contents**
Negative Control	1 x 1.5mL	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide <0.1% (preservative)
Positive Control	1 x 1.5mL	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide <0.1% (preservative)
Cut-off Calibrator	1 x 1.5mL	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide <0.1% (preservative)

Calibrators	6 x 1.5mL	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide <0.1% (preservative)
Conjugate, IgG	1 x 15mL	Blue	Blue	Containing: Anti-human Immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15mL	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15mL	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				

4. Materials Required but Not Provided

- Microtiter plate reader with 450 nm reading filter and optional 620 nm reference filter (600-690 nm)
- Glassware (cylinder 100 - 1000mL), test tubes for dilutions
- Vortex mixer
- Precision pipettes (10, 100, 200, 500, 1000 µL) or adjustable multi-pipette (100 - 1000 µL)
- Microplate washing device (multichannel pipette or automated system)
- Absorbent paper
- Timer
- The assay is designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.)

5. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

6. Precautions of Use

6.1 Health hazard data

Only trained staff should use the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following information for maximum safety.

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified as

being an irritant to eyes and skin it is recommended to avoid contact with eyes and skin and to wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by the CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HBsAg, Hepatitis C, and HIV-1. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle kit controls, standards and samples as if capable of transmitting infectious diseases and according to local and national requirements.

6.2 General directions for use

- If product information, including the labeling, is defective or incorrect, please contact ALPCO.
- Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.
- Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for optimal performance of the test.
- **Incubation: It is recommended to run automated test systems at 30°C/86°F.**
- Never expose components to higher temperature than 37°C/98.6°F.
- Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips previously used with other reagents.

7. Sample Collection, Handling and Storage

It is preferable to use freshly collected serum samples. Blood draw must follow local and national requirements. Do not use icteric, lipemic, hemolyzed, or bacterially contaminated samples. Sera with particulates should be cleared by low-speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry, and empty tubes.

After separation, the serum samples should be used within 8 hours, stored tightly closed at 2-8°C/35-46°F for up to 48 hours, or frozen at -20°C/-4°F for longer periods.

8. Assay Procedure

8.1 Preparations prior to pipetting

Reagents:
Dilute concentrated reagents:

- Dilute the concentrated sample buffer 1:5 with distilled water (e.g., 20 mL plus 80 mL).
- Dilute the concentrated wash buffer 1:50 with distilled water (e.g., 20 mL plus 980 mL).
- It is recommended to mark the caps of the different calibrators to avoid mistakes.

Samples:
Dilute serum samples 1:101 with sample buffer (1x)
e.g., 1000 µL sample buffer (1x) + 10 µL serum. Mix well!

Washing:
Prepare 20 mL of 1X working wash buffer per 8 wells or 200 mL for 96 wells
e.g., 4 mL concentrate plus 196 mL distilled water.

Automated washing:
Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:
Discard liquid from wells by inverting the plate. Knock the microwell frame vigorously with wells down on clean absorbent paper. Pipette 300 µL of 1X working wash buffer into each well, wait 20 seconds. Repeat the whole procedure two more times for a total of 3 wash cycles.

Microplates:
Calculate the number of wells required for test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

8.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For *QUANTITATIVE* interpretation

	1	2	3	4...
A	Cal A	Cal E	S1	
B	Cal A	Cal E	S1	
C	Cal B	Cal F	S2	
D	Cal B	Cal F	S2	
E	Cal C	PC	S3	
F	Cal C	PC	S3	
G	Cal D	NC	…	
H	Cal D	NC	…	

For *QUALITATIVE* interpretation

	1	2	3	4...
A	NC	S2		
B	NC	S2		
C	CC	S3		
D	CC	S3		
E	PC	…		
F	PC	…		
G	S1	…		
H	S1	…		

CalA: calibrator A
CalB: calibrator B
CalC: calibrator C
CalD: calibrator D
CalE: calibrator E
CalF: calibrator F
PC: positive control
NC: negative control
CC: cut-off calibrator
S1: sample 1
S2: sample 2
S3: sample 3

9. Assay Steps

1) Ensure preparations from step 8.1 above have been carried out prior to pipetting.
2) Pipette 100 µL of either:
   a) Calibrators (CAL.A to CAL.F) for *SEMI-QUANTITATIVE interpretation* or
   b) Cut-off Calibrator (CC) for *QUALITATIVE interpretation* and 100 µL of each of the following:
      - Negative Control (NC)
      - Positive Control (PC)

3) Pipette 100 µL of diluted samples into the designated microwells (S1, S2 . . .)
4) Incubate for 30 minutes at 20-32°C/68-89.6°F.
5) Wash 3 times with 300 µL 1X working wash buffer.
6) Pipette 100 µL conjugate into each well.
7) Incubate for 30 minutes at room temperature (20-32°C/68-89.6°F).
8) Wash 3 times with 300 µL 1X working wash buffer.
9) Pipette 100 µL TMB substrate into each well.
10) Incubate for 30 minutes at 20-32°C/68-89.6°F, in the dark.
11) Pipette 100 µL stop solution into each well, using the same order as pipetting the substrate.
12) Incubate 5 minutes minimum.
13) Agitate plate carefully for 5 seconds by tapping the plate frame.
14) Read absorbance at 450 nm (450/620 nm, if possible) within 30 minutes.

10. Calculation of Results

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in U/mL (x-axis). For best results it is recommended to use log/lin coordinates and 4-parameter fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/mL.

Negative Range	Equivocal Range	Positive Range
≤ 12 U/mL	12-18 U/mL	> 18 U/mL

Example of a standard curve – do not use for interpretation of results
It is recommended to pipette calibrators in parallel for each run.

Calibrators IgG	***OD 450/620 nm***	***CV % (Variation)***
0 U/mL	0.049	0.0
3 U/mL	0.164	2.6
10 U/mL	0.332	2.5
30 U/mL	0.675	0.9
100 U/mL	1.387	0.0
300 U/mL	2.272	0.5

Example of calculation

Sample	**Replicate (OD)**	**Mean (OD)**	**Result (U/mL)**
S 1	0.763/0.787	0.775	37.3
S 2	1.053/1.039	1.046	61.5

Samples above the highest calibrator should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as <Min.

For lot-specific data, see enclosed quality control leaflet. Laboratories may utilize in-house quality controls and/or internal pooled sera, as determined by local or national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment, and sample population according to their own established procedures.

If the values of the controls do not meet the criteria the test is invalid and has to be repeated. The following technical issues should be verified: expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause, please contact ALPCO.

For **qualitative interpretation**, read the optical density of the cut-off calibrator and the samples. Compare sample ODs with the OD of the cut-off calibrator. For qualitative interpretation we recommend considering sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative = Sample OD < 0.8 x OD Cut-off
Equivocal = Sample OD is ≤ 1.2 x OD Cut-off and ≥ 0.8 x OD cut-off
Positive = Sample OD > 1.2 x OD Cut-off

11. Technical Data

Sample material:	serum
Sample volume:	10 µL of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at room temperature (20-32°C/68-89.6°F)
Calibration range:	0 - 300 U/mL
Analytical sensitivity:	1.0 U/mL
Storage:	2-8°C/35-46°F use original vials only
Number of determinations:	96 tests

12. References

1. Witte T, Matthias T, Arnett FC, Peter HH, Hartung K, Sachse C, Wigand R, Braner A, Kalden JR, Lakomek HJ, Schmidt RE (2000). IgA and IgG autoantibodies against alpha-fodrin as markers for Sjogren‘s syndrome and systemic lupus erythematosus. J Rheumatol 27 (11):2617-20.

2. Haneji N, Nakamura T, Takio K, Yanagi K, Higashiyama H, Saito I, Noji S, Sugino H, Hayashi Y (1997). Identification of alpha-fodrin as a candidate autoantigen in primary Sjogren‘s syndrome. Science 276 (5312):604-7.

3. Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, et al. (1993). Preliminary criteria for the classification of Sjogren‘s syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum 36 (3):340-7.