Anti-DFS70 ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EA 159z-9601 G	DFS70 (dense fine speckles 70 antigen)	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative in vitro assay for human autoantibodies of the IgG class against DFS70 (dense fine speckles 70 antigen) in serum or plasma for the diagnosis of autoimmune diseases.

Application: Anti-DFS70 antibodies have been associated (in the literature) with various diseases, for example atopic dermatitis, Vogt-Harada syndrome and asthma, although they are not specific for these diseases.
If anti-DFS70 antibodies are present, a systemic rheumatic autoimmune disease (SRA) is unlikely. However, a thorough ANA differentiation is essential even if these antibodies are detected since the presence of a SRA cannot be completely excluded. A positive DFS70 finding may be helpful in clarifying ANA patterns that cannot be attributed to any disease-specific autoantibodies.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with dense fine speckles 70 antigen. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

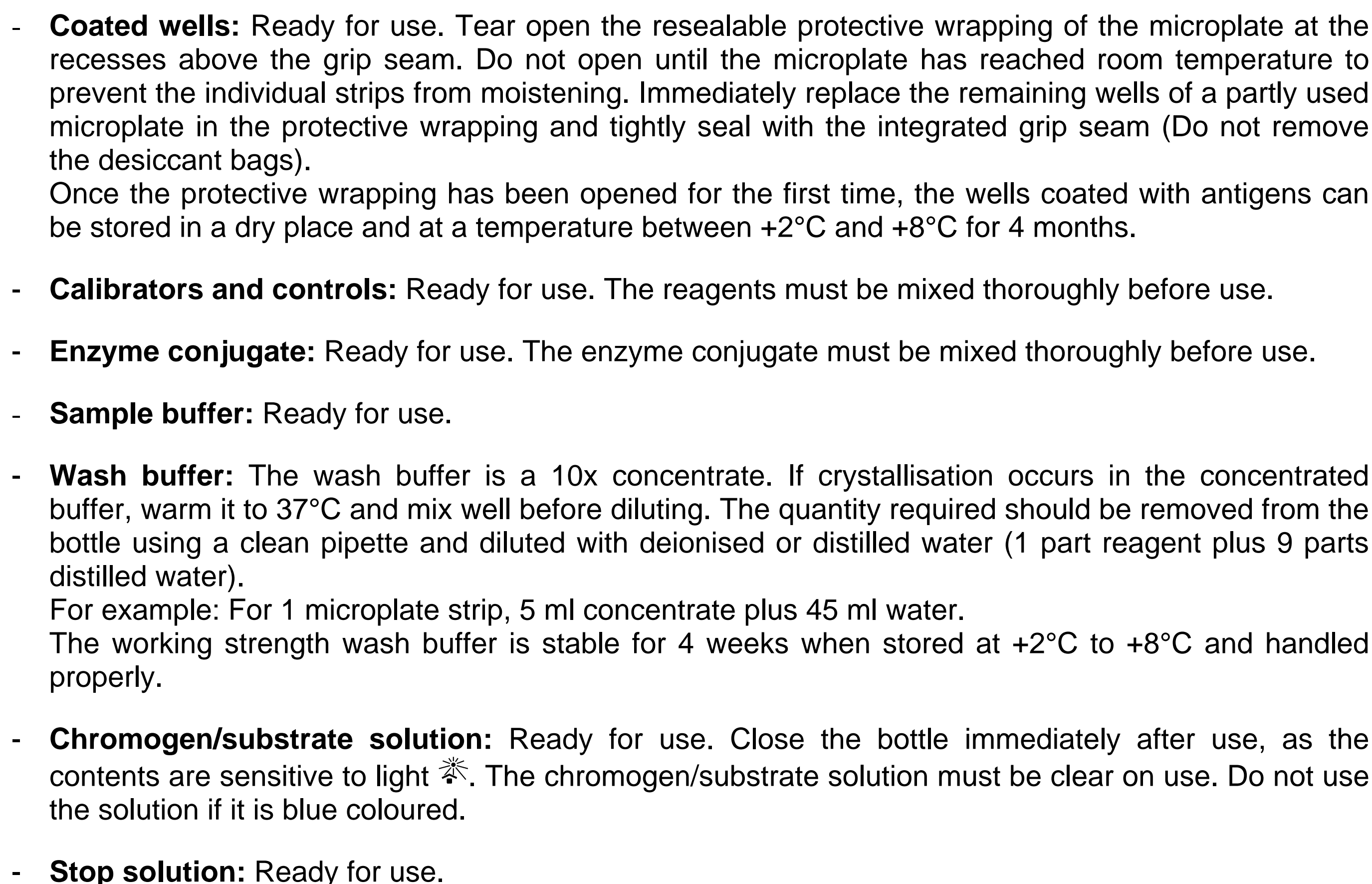
Contents of the test kit:

Component	Colour	Format	Symbol
1. **Microplate wells coated with antigens** 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. **Calibrator** (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL
3. **Positive control** (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. **Negative control** (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. **Enzyme conjugate** peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
6. **Sample buffer** ready for use	violet	1 x 100 ml	SAMPLE BUFFER
7. **Wash buffer** 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. **Chromogen/substrate solution** TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. **Stop solution** 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. **Test instruction**	---	1 booklet	
11. **Quality control certificate**	---	1 protocol	

LOT	Lot description	CE	Storage temperature
IVD	In vitro diagnostic medical device		Unopened usable until

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.



- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bags).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ☀. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples to be investigated are diluted **1:201** with sample buffer.
Example: Add 5 µl of sample to 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

Sample incubation: (1st step)

Transfer 100 µl of the calibrator, positive or negative control or diluted patient samples into the individual microplate wells according to the pipetting protocol.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:

Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping the reaction:

Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I and the Analyzer I-2P and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C**	**P 6**	**P 14**	**P 22**								
B	**pos.**	**P 7**	**P 15**	**P 23**								
C	**neg.**	**P 8**	**P 16**	**P 24**								
D	**P 1**	**P 9**	**P 17**									
E	**P 2**	**P 10**	**P 18**									
F	**P 3**	**P 11**	**P 19**									
G	**P 4**	**P 12**	**P 20**									
H	**P 5**	**P 13**	**P 21**									

The pipetting protocol for microtiter strips is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrator (C), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0:	**negative**
Ratio ≥1.0:	**positive**

For diagnosis, the clinical findings of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against DFS70, results are provided in the form of ratio which are a relative measure for the concentration of antibodies in patient samples.

For every group of tests performed, the ratio values determined for the calibrator and for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrator is subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: DFS70 is used as a recombinant antigen.

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-DFS70 ELISA (IgG) is ratio 0.04.

Cross reactivity: This ELISA specifically detects autoantibodies of class IgG against DFS70 antigen. Cross reactions with other autoantibodies were not found.

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 3 determinations performed in 10 different test runs.

Intra-assay variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	1.0	4.1
2	5.7	2.6
3	6.1	2.3

Inter-assay variation, n = 3 x 10		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	1.2	5.6
2	5.7	3.5
3	5.3	4.4

Prevalence: The prevalence of anti-DFS70 antibodies was investigated in 99 clinically characterised patients with connective tissue disease and a panel of 200 healthy blood donors, using the EUROIMMUN Anti-DFS70 ELISA (IgG). The following results were obtained:

Patient group	**n**	**Anti-DFS70 positive**
Sjögren's syndrome	25	0 (0%)
Systemic lupus erythematosus	27	4 (14,8%)
Progressive systemic sclerosis	27	3 (11,1%)
Mixed connective tissue disease	3	1 (33,3%)
Undifferentiated connective tissue disease	17	4 (23,5)
Blood donors I	200	6 (3%)

Sensitivity and specificity: 50 samples from a panel with patients suffering from rheumatoid diseases who showed positive, partly unclear ANA patterns, were investigated with the EUROIMMUN Anti-DFS70 ELISA (IgG), and the EUROLINE ANA Profile 3 plus DFS70 (IgG) as a reference method. The determined analytical sensitivity of the ELISA amounted to 92.3% and the analytical specificity to 100%.

n = 50		**EUROLINE ANA Profile 3 plus DFS70 (IgG)**	
		positive	negative
Anti-DFS70 ELISA (IgG)	positive	24	0
	negative	2	24

Reference range: Anti-DFS70 antibody levels were determined with the EUROIMMUN ELISA in 200 samples of healthy blood donors (blood donors II). The medium concentration of antibodies against DFS70 (IgG) amounted to 0.3 ratio and encompassed a range from 0.1 to 3.5 ratio. At a cut-off of ratio 1.0, 2.5% of the blood donors were anti-DFS70 positive.

Cut-off	Percentile
0.8 Ratio	97.
1.1 Ratio	98.
1.5 Ratio	99.

Clinical significance

Antibodies against nuclear antigens (ANA) are directed against various cell nuclear components. Among the most important nuclear antigens, including cytoplasmic antigens, are nRNP/Sm, Sm, SS-A (Ro), SS-B (La), Scl-70, PM-Scl, Jo-1, centromeres, PCNA, dsDNA, nucleosomes, histones and ribosomal P-proteins. They are mainly components of functional nuclear particles, are bound to nucleic acids or fulfil functions in the cell cycle, e.g. in transcription or translation.

The investigation of ANA and subsequent differentiation within the ANA (or ENA, extractable nuclear antigens) spectrum contributes greatly to establishing a diagnosis, particularly in the following rheumatic diseases:
- Systemic lupus erythematosus (SLE),
- Sharp syndrome (mixed connective tissue disease = MCTD),
- Sjögren's syndrome (SS),
- Systemic sclerosis (SSc), and
- Poly-/dermatomyositis (PM/DM).

The antigen **DFS70** (dense fine speckles 70 antigen, 70 kDa) is identical to the transcription coactivator p75 and the lens epithelium-derived growth factor p75 (LEDGF/p75).

Autoantibodies against DFS70 can be determined in several autoimmune diseases (e.g. atopic dermatitis, asthma, Vogt-Harada syndrome and interstitial cystitis, as well as in rheumatic diseases) with low prevalence, and sometimes also in healthy blood donors (up to 8%).

The diagnostic value of DFS70 autoantibodies for the exclusion or confirmation of a systemic rheumatic autoimmune disease (SRA) is low. After a diligent ANA differentiation, either rheumatologically relevant ANA indicating an SRA are detected, or not, which makes the presence of an SRA rather unlikely. A positive or negative finding for anti-DFS70 does not change the finding. In ANA investigation using IIFT, a diligent monospecific ANA differentiation needs to be conducted in any case without exceptions, independently of whether antibodies against DFS70 are present or not. Only if no disease-relevant ANA are detected after the specialised diagnostics can a positive DFS70 result help to explain the observed IIFT pattern (frequently: fine granular with positive chromosome regions).

Literature references

1. Ganapathy V, Casiano CA. **Autoimmunity to the nuclear autoantigen DFS70 (LEDGF): what exactly are the autoantibodies trying to tell us?** Arthritis Rheum 50 (2004) 684-688.
2. EUROIMMUN AG. Stöcker W, Schlumberger W, Krüger C. Alle Beiträge zum Thema Autoimmundiagnostik und Labordiagnostik der Infektionskrankheiten. In: Gressner A, Arndt T (Hrsg.) **Lexikon der Medizinischen Laboratoriumsdiagnostik.** 2. Auflage. Springer Medizin Verlag, Heidelberg (2012).
3. Mahler M, Fritzler MJ. **The clinical significance of the dense fine speckled immunofluorescence pattern on HEp-2 cells for the diagnosis of systemic autoimmune diseases.** Clin Dev Immunol 1155 (2012) 494356.
4. Mahler M, Parker T, Peebles CL, Andrade LE, Swart A, Carbone Y, Ferguson DJ, Villalta D, Bizzaro N, Hanly JG, Fritzler MJ. **Anti-DFS70/LEDGF antibodies are more prevalent in healthy individuals compared to patients with systemic autoimmune rheumatic diseases.** J Rheumatol 39 (2012) 2104-2110.
5. Miyara M, Albesa R, Charuel JL, El Amri M, Fritzler MJ, Ghillani-Dalbin P, Amoura Z, Musset L, Mahler M. **Clinical phenotypes of patients with anti-DFS70/LEDGF antibodies in a routine ANA referral cohort.** Clin Dev Immunol 1155 (2013) 703759.
6. Muro Y, Sugiura K, Morita Y, Tomita Y. **High concomitance of disease marker auto-antibodies in anti-DFS70/LEDGF autoantibody-positive patients with autoimmune rheumatic disease.** Lupus 17 (2008) 171-176.
7. Watanabe A, Kodera M, Sugiura K, Usuda T, Tan EM, Takasaki Y, Tomita Y, Muro Y. **Anti-DFS70 antibodies in 597 healthy hospital workers.** Arthritis Rheum 50 (2004) 892-900.