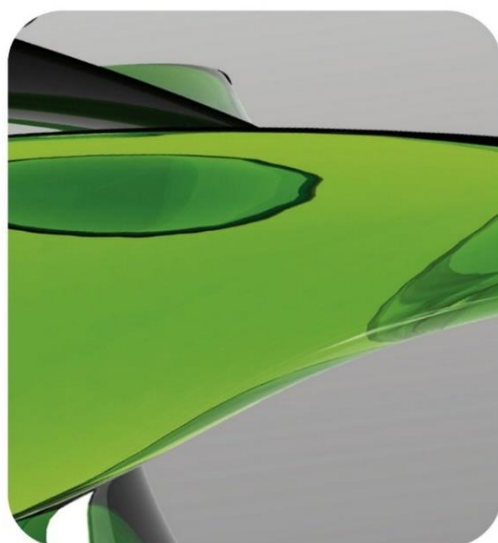




AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS



AESKULISA[®]

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA[®] dsDNA-G

REF 30-7142US





Instruction manual

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1 Intended Use

AESKULISA® dsDNA-G is a solid phase enzyme immunoassay with human recombinant double-stranded DNA (dsDNA) for the quantitative and qualitative detection of IgG antibodies against dsDNA in human serum.

The assay is a tool in the diagnosis of systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.

2 Clinical Application and Principle of the Assay

Antibodies binding to DNA belong to the group of anti-nuclear antibodies (ANA) that have been observed in several autoimmune diseases. Antibodies reacting with native double-stranded (ds) DNA are regarded as being specific for systemic lupus erythematosus (SLE) and have been observed in approximately 50-80% of the patients. Anti-dsDNA antibodies mainly recognize the phosphat units of the DNA, thus these autoantibodies also bind single stranded DNA (ssDNA). To ensure correct quantitation of anti-dsDNA antibodies the used antigen has been proven to be free of contamination with ssDNA.

Antibodies against dsDNA are found during active phases of SLE. The amount of the serum concentration is positively correlated with the severity of the disease. Thus, detection of these autoantibodies is important for the diagnosis and the clinical monitoring of SLE. Consequently, it has been established as 1 of the 11 ACR-criteria for the diagnosis of SLE. Most patients with SLE display IgG class antibodies against dsDNA. These autoantibodies are associated with lupus nephritis. Approximately 30% of the SLE patients develop IgA class anti-dsDNA antibodies, additionally. There have been suggestions that the presence of these IgA class anti-dsDNA antibodies may define a certain subset of SLE patients. Indeed studies demonstrated the association of this subclass with certain parameters of the disease activity, such as elevated erythrocyte sedimentation rate, or the consumption of complement component C3, as well as the clinical parameters of cutaneous vasculitis, acral necrosis and erythema. While no association was found for nephritis and arthritis.

IgM class anti-dsDNA antibodies were found in 52% of the sera from patients with SLE. In contrast to IgG and IgA class autoantibodies, the subclass IgM antibodies do not correlate with disease activity. However, a highly significant negative correlation between IgM anti-dsDNA antibodies and lupus nephritis, including its laboratory parameters was demonstrated. Therefore, IgM class anti-dsDNA antibodies may indicate a subset of lupus patients being protected against the risk of developing nephritis.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the Specific antigen. Patient's antibodies, if present in the specimen, 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 rate 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 patient sample.



3 Kit Contents

To be reconstituted:

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide < 0.1% (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide < 0.1% (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Cut-off Control 1 vial, 1.5 ml (capped blue: yellow solution)
Containing: Human serum (diluted), sodium Azide < 0.1% (preservative)

Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 IU/ml
(color increasing with concentration: yellow solutions)
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Conjugate 1 vial, 15 ml IgG (capped blue: blue solution)
Containing: goat Anti-human immunoglobulins conjugated to horseradish peroxidase (preservative)

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H₂O₂

Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells
Coating see paragraph 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm filter (600-690nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia and the European Pharmacopeia.

4 Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35.6-46.4°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 2-8°C/35.6-46.4°F, at least.

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.



Product Ref.	30-7142US
Product Desc.	dsDNA-G
Manual Rev. No.	011: 2026-01-14

5 Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though the kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

WARNING! Calibrators, Controls and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or adsorbed by skin or eyes. NaN₃ 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 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 FDA 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 patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

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 (20-26°C/68-78.8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

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 used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic and medicinal methods if the patient has got infectious diseases accompanied by medication.

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles 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 immediately, respectively stored tightly closed at 2-8°C/35.6-46.4°F up to three days, or frozen at -20°C/-4°F for longer periods.

7 Assay Procedure

7.1 Preparations prior to pipetting

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).

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 diluted wash buffer (1x) 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 with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35.6-46.4°F).

7.2 Work flow

For pipetting scheme see Annex A, for the test procedure see Annex B

- Pipette 100 µl of each patient's diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off control and negative and positive controls into the designated wells.
- Incubate for 30 minutes at room temperature (20-26°C/68-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at room temperature (20-26°C/68-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at room temperature (20-26°C/68-78.8°F), in the dark.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8 Quantitative and Qualitative Interpretation

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 **IU/ml (x-axis)**. For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in **IU/ml**.

Normal Range

≤ 15 IU/ml

Positive Results

> 15 IU/ml

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgG		OD 450/620 nm	CV % (Variation)
0	IU/ml	0.036	2.9
3	IU/ml	0.176	2.3
10	IU/ml	0.314	2.9
30	IU/ml	0.618	2.9
100	IU/ml	1.312	0.1
300	IU/ml	2.076	0.7

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.799/0.744	0.722	40.3
P 02	1.404/1.393	1.399	119.5

For lot specific data, see enclosed quality control certificate. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

Do not use this example for interpreting patients results!

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

For **qualitative interpretation** read the optical density of the cut-off control and the patient samples. Compare patient's OD with the OD of the cut-off control. All samples which are higher than cut-off are considered positive.

Negative: OD patient < OD_{cut-off}
Positive: OD patient > OD_{cut-off}

9 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 (20-26°C/68-78.8°F)
Calibration range:	0-300 IU/ml
Analytical sensitivity:	3.0 IU/ml
Storage:	at 2-8°C/35.6-46.4°F use original vials, only
Number of determinations:	96 tests

10 Performance Data

10.1 Analytical sensitivity

The detection limit of **AESKULISA® dsDNA-G** has been found at 3 IU/ml.

10.2 Specificity and sensitivity

The microplate is coated with **recombinant human dsDNA**. No cross reactivities to other autoantigens have been found. For determination of sensitivity and specificity sera of 54 patients suffering from SLE have been tested with the **AESKULISA® dsDNA-G** and an already marketed predicate device. Also 80 healthy donors were tested on the **AESKULISA® dsDNA-G** and were all shown to be negative.

		predicate device		
		positive	non-positive	Total
AESKULISA®	positive	34	13	47
	negative	0	7	7
	Total	34	20	54

clinical specificity: 80 / 80= 100%

clinical sensitivity: 47 / 54= 87%

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	measured concentration (IU/ml)	expected concentration (IU/ml)	Recovery (%)
1	1 / 100	42.9	43.2	99.3
	1 / 200	20.4	21.6	99.4
	1 / 400	9.3	10.8	86.1
	1 / 800	4.9	5.4	90.7
2	1 / 100	179.4	176.0	101.9
	1 / 200	86.4	88.0	98.2
	1 / 400	41.8	44.0	95.0
	1 / 800	19.8	22.0	90.0



10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intra-Assay			Inter-Assay		
Sample No.	Mean (U/ml)	CV (%)	Sample No.	Mean (U/ml)	CV (%)
1	199.88	1.0	1	463.3	2.6
2	80.14	1.68	2	171.6	2.3
3	12.71	5.91	3	13.2	5.9

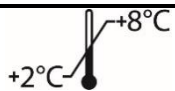
10.5 Calibration

The **AESKULISA® dsDNA-G** is calibrated against the WHO reference serum lupus (oligo-specific) anti-dsDNA antibodies. The results are expressed in IU/ml.

11 Literature

- Tan EM, Cohen AS, Fries JF, et al. (1982).**
Revised criteria for the classification of systemic lupus erythematosus.
Arthritis Rheumatism 25: 1271-1277.
- Witte T, Hartung K, Matthias T, Sachse C, Fricke M, Deicher H, Kalden JR, Lakomek HJ, Peter HH, Schmidt RE (1998).**
Association of IgA anti-dsDNA antibodies with vasculitis and disease activity in systemic lupus erythematosus.
Rheumatol Int 18: 63-69.
- Witte T, Hartung K, Matthias T, Sachse C, Fricke M, Deicher H, Kalden JR, Lakomek HJ, Peter HH, Schmidt RE, SLE study group (1998).**
IgM anti-dsDNA antibodies in systemic lupus erythematosus: negative association with nephritis.
Rheumatol Int 18: 85-91.

12 Regulatory Symbols

IVD	For in vitro diagnostic use
REF	Catalog number
LOT	Lot
UDI	Unique Device Identifier
	96 tests
	See instructions for use
	Use by
	Store at 2-8°C (35.6-46.4°F)
	Manufactured by
CON +	Positive Control
CON -	Negative Control
CAL	Calibrator
CO-CAL	Cut off Calibrator
CONJ	Conjugate
MP	Coated microtiter plate
WASHB 50x	Wash buffer
SUB	Substrate buffer
STOP	Stop solution
SB 5x	Sample buffer
Rx only	For Prescription Use only

Annex

A: Pipetting scheme

We suggest pipetting calibrators, controls and samples as follows:

For **quantitative interpretation** use calibrators to establish a standard curve

For **qualitative interpretation** use cut-off control

	for quantitative interpretation use calibrators to establish a standard curve						for qualitative interpretation use cut-off control					
	1	2	3	4	5	6	7	8	9	10	11	12
A	CalA	CalE	P1				NC	P2				
B	CalA	CalE	P1				NC	P2				
C	CalB	CalF	P2				CC	P3				
D	CalB	CalF	P2				CC	P3				
E	CalC	PC	P3				PC	...				
F	CalC	PC	P3				PC	...				
G	CalD	NC	...				P1	...				
H	CalD	NC	...				P1	...				

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 control

P1: patient 1

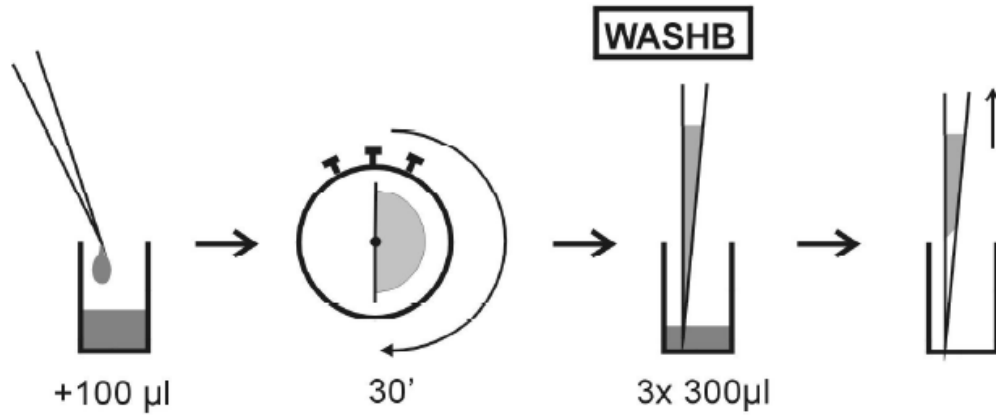
P2: patient 2

P3: patient 3

B: Test Procedure

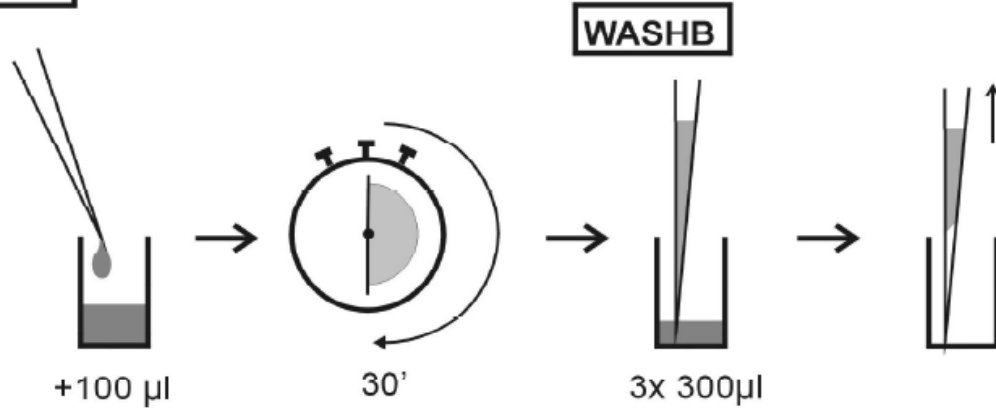
Samples (1:101) / Controls

1



CONJ

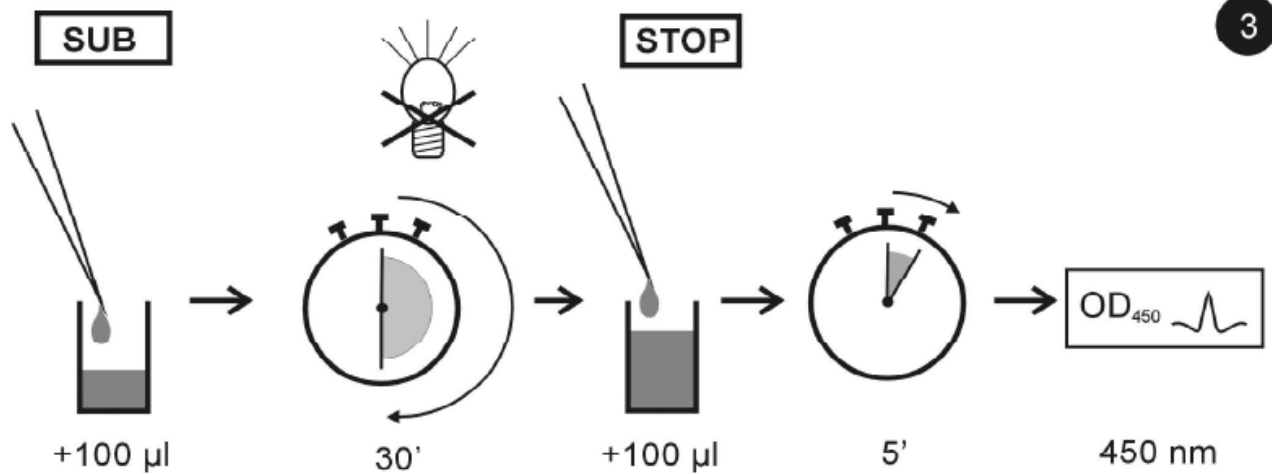
2



SUB

STOP

3



C: Test Protocol

Assay/Test: _____ Incubation/ Inkub. : 1. _____ min Date/Datum: _____
 Temperature/Temperatur: _____ °F _____ °C Signature/Unterschrift: _____
 Name: _____ 2. _____ min
 3. _____ min

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												