Anti-ss-DNA Screen (IgA, IgG, IgM) ELISA

For the quantitative/qualitative determination of IgA/G/M against single stranded DNA in serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 35-SSSHU-E01
Size: 96 wells
1. Intended Use

AESKULISA ssDNA-Check is a solid phase enzyme immunoassay with human recombinant single-stranded DNA (ssDNA) for the combined quantitative and qualitative detection of IgA/G/M antibodies against ssDNA in human serum. Antibodies against ssDNA mainly recognize its basic compound, which is masked inside the helical structure of native DNA.

The assay is a tool in the differential diagnosis of systemic lupus erythematosus (SLE).

2. Clinical Application and Principle of the Assay

Antibodies binding to DNA belong to the group of anti-nuclear antibodies (ANA) which occur in several autoimmune diseases. Antibodies reacting with native double-stranded (ds) DNA are regarded as being specific for systemic lupus erythematosus (SLE) and appear in approximately 50-80% of these patients. Antibodies against dsDNA are found during active phases of SLE, their serum concentration correlates with the severity of the disease. Thus, detection of these autoantibodies is important for the diagnosis and clinical monitoring of the disease and consequently has been established as 1 of the 11 ACR-criteria for the diagnosis of SLE.

Antibodies to single-stranded (ss) DNA are found with a frequency of up to 87% during acute phases and 43% during inactive phases in patients with SLE. They are not specific for SLE as they may be found in other diseases like infectious mononucleosis (40%), autoimmune hepatitis (58%), acute myeloid leukemia (89%), acute lymphatic leukemia, chronic myeloid leukemia (60%) and juvenile rheumatoid arthritis (35-50%), too.

SLE-like symptoms may be induced by drugs, too. As anti-ssDNA antibodies are found with a frequency of up to 50 % in patients with a drug-induced LE, the determination of antibodies against ssDNA is very helpful for the differential diagnosis.

**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 20, 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: colorless solution)  
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Cut-off Calibrator 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 U/ml  
(color increasing with concentration: yellow solutions)  
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Conjugate  1 vial 15ml IgA/G/M (capped white: red solution)  
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

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 reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000ml). Microplate washing device (300 µl repeating or multi-channel 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 (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

4. 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 for 1 month at 4°C/39°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.**
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 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 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 (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation:** We recommend test performance at 30°C/86°F for automated systems.

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 methods.**

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/35-46°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/35-46°F).

7.2 Work flow

For pipetting scheme see Annex A, for the test procedure see Annex B
We recommend pipetting samples and calibrators in duplicate.
Cut-off calibrator should be used for qualitative testing only.

- Pipette 100 µl of each patient's diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off calibrator and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
- 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 **U/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 **U/ml**.

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>Equivocal Range</th>
<th>Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 16 U/ml</td>
<td>16 - 24 U/ml</td>
<td>&gt;24 U/ml</td>
</tr>
</tbody>
</table>

**Example of a standard curve**
We recommend pipetting calibrators in parallel for each run.

<table>
<thead>
<tr>
<th>Calibrators IgA/G/M</th>
<th>OD 450/620 nm</th>
<th>CV % (Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 U/ml</td>
<td>0.030</td>
<td>3.1</td>
</tr>
<tr>
<td>3 U/ml</td>
<td>0.143</td>
<td>2.9</td>
</tr>
<tr>
<td>10 U/ml</td>
<td>0.297</td>
<td>1.4</td>
</tr>
<tr>
<td>30 U/ml</td>
<td>0.692</td>
<td>3.0</td>
</tr>
<tr>
<td>100 U/ml</td>
<td>1.300</td>
<td>0.3</td>
</tr>
<tr>
<td>300 U/ml</td>
<td>2.200</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Example of calculation**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Replicate (OD)</th>
<th>Mean (OD)</th>
<th>Result (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 01</td>
<td>1.267/1.253</td>
<td>1.260</td>
<td>91.6</td>
</tr>
<tr>
<td>P 02</td>
<td>0.583/0.573</td>
<td>0.578</td>
<td>25.1</td>
</tr>
</tbody>
</table>

For lot specific data, see enclosed quality control leaflet. 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 calibrator and the patient samples. Compare patient’s OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider 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:** \( \text{OD}_{\text{patient}} < 0.8 \times \text{OD}_{\text{cut-off}} \)

**Equivocal:** \( 0.8 \times \text{OD}_{\text{cut-off}} \leq \text{OD}_{\text{patient}} \leq 1.2 \times \text{OD}_{\text{cut-off}} \)

**Positive** \( \text{OD}_{\text{patient}} > 1.2 \times \text{OD}_{\text{cut-off}} \)
9. Technical Data

<table>
<thead>
<tr>
<th>Sample material:</th>
<th>serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample volume:</td>
<td>10 µl of sample diluted 1:101 with 1x sample buffer</td>
</tr>
<tr>
<td>Total incubation time:</td>
<td>90 minutes at 20-32°C/68-89.6°F</td>
</tr>
<tr>
<td>Calibration range:</td>
<td>0-300 U/ml</td>
</tr>
<tr>
<td>Analytical sensitivity:</td>
<td>1.0 U/ml</td>
</tr>
<tr>
<td>Storage:</td>
<td>at 2-8°C/35-46°F use original vials, only</td>
</tr>
<tr>
<td>Number of determinations:</td>
<td>96 tests</td>
</tr>
</tbody>
</table>

10. Performance Data

10.1 Analytical sensitivity
Testing sample buffer 30 times on AESKULISA ssDNA-Check gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and sensitivity
The microplate is coated with recombinant human ssDNA. No crossreactivities to other autoantigens have been found. Antibodies against ssDNA occur in different diseases which are shown in the table below:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE (acute phase)</td>
<td>87%</td>
</tr>
<tr>
<td>SLE (inactive phase)</td>
<td>43%</td>
</tr>
<tr>
<td>infectious mononucleosis</td>
<td>40%</td>
</tr>
<tr>
<td>acute myeloid leukemia</td>
<td>89%</td>
</tr>
<tr>
<td>chronic myeloid leukemia</td>
<td>60%</td>
</tr>
<tr>
<td>autoimmune hepatitis</td>
<td>58%</td>
</tr>
<tr>
<td>juvenile rheumatoid arthritis</td>
<td>35-50%</td>
</tr>
</tbody>
</table>

The data has been aquired with the AESKULISA ssDNA-Check (REF7144).

Correlation:

The comparability of performance data was assessed with at least 30 sera tested on both, AESKULISA 7144 and AESKULISA 3144. A linear regression analysis of the two products showed that the two products are equivalent. Data can be received upon request.
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.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Dilution Factor</th>
<th>measured concentration (U/ml)</th>
<th>expected concentration (U/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 / 100</td>
<td>68.9</td>
<td>70.0</td>
<td>98.4</td>
</tr>
<tr>
<td></td>
<td>1 / 200</td>
<td>33.5</td>
<td>35.0</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>1 / 400</td>
<td>16.8</td>
<td>17.5</td>
<td>96.0</td>
</tr>
<tr>
<td></td>
<td>1 / 800</td>
<td>8.4</td>
<td>8.8</td>
<td>95.5</td>
</tr>
<tr>
<td>2</td>
<td>1 / 100</td>
<td>47.6</td>
<td>48.0</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>1 / 200</td>
<td>23.1</td>
<td>24.0</td>
<td>96.3</td>
</tr>
<tr>
<td></td>
<td>1 / 400</td>
<td>11.5</td>
<td>12.0</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>1 / 800</td>
<td>5.9</td>
<td>6.0</td>
<td>98.3</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (U/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>127.0</td>
<td>2.6</td>
</tr>
<tr>
<td>2</td>
<td>63.0</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>42.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Inter-Assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>125.0</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>60.0</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>45.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

10.5 Calibration
Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11. Literature

### 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 calibrator.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>CalA</td>
<td>CalE</td>
<td>P1</td>
<td></td>
<td></td>
<td></td>
<td>NC</td>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>CalA</td>
<td>CalE</td>
<td>P1</td>
<td></td>
<td></td>
<td></td>
<td>NC</td>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>CalB</td>
<td>CalF</td>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td>CC</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>CalB</td>
<td>CalF</td>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td>CC</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>E</strong></td>
<td>CalC</td>
<td>PC</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td>PC</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>CalC</td>
<td>PC</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td>PC</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>G</strong></td>
<td>CalD</td>
<td>NC</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td>P1</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>CalD</td>
<td>NC</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td>P1</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PC: positive control  
NC: negative control  
CC: Cut-off calibrator  
P1: patient 1  
P2: patient 2  
P3: patient 3
Annex B: Test Procedure

1. Samples (1:101) / Controls

   \[+100 \mu l \rightarrow 30' \rightarrow 3x 300 \mu l\]

2. CONJ

   \[+100 \mu l \rightarrow 30' \rightarrow 3x 300 \mu l\]

3. SUB

   \[+100 \mu l \rightarrow 30' \rightarrow +100 \mu l \rightarrow 5' \rightarrow OD_{450}\]

   \[450 \text{ nm}\]