IDK® DAO ELISA

For the in vitro determination of DAO in serum and dried blood spots

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1. INTENDED USE
This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of diamine oxidase (DAO) in serum and dried blood spots. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION
Diamine oxidase (DAO) is a body’s own enzyme that metabolises histamine. Although DAO is found practically in the whole body, the most important site of its action is the intestine. The enzymatic activity of DAO determines the histamine degradation speed.

Our IDK® DAO ELISA kit is intended for determination of the diamine oxidase (DAO) concentration in serum.

3. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Cat. No</th>
<th>Label</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>KR8500</td>
<td>PLATE</td>
<td>Microtiter plate, pre-coated</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>KR8500</td>
<td>WASHBUF</td>
<td>Wash buffer concentrate, 5 x</td>
<td>4 x 100 ml</td>
</tr>
<tr>
<td>KR8500</td>
<td>STD</td>
<td>Standards, lyophilised (see specification for concentrations)</td>
<td>4 x 5 vials</td>
</tr>
<tr>
<td>KR8500</td>
<td>CTRL1</td>
<td>Control, lyophilised (see specification for range)</td>
<td>4 x 1 vial</td>
</tr>
<tr>
<td>KR8500</td>
<td>CTRL2</td>
<td>Control, lyophilised (see specification for range)</td>
<td>4 x 1 vial</td>
</tr>
<tr>
<td>KR8500</td>
<td>AB</td>
<td>Detection antibody concentrate, biotinylated</td>
<td>1 x 200 µl</td>
</tr>
<tr>
<td>KR8500</td>
<td>CONJ</td>
<td>Conjugate concentrate, peroxidase-labelled (streptavidin)</td>
<td>1 x 200 µl</td>
</tr>
<tr>
<td>KR8500</td>
<td>ABBUF</td>
<td>Dilution buffer for AB and CONJ, ready-to-use</td>
<td>1 x 50 ml</td>
</tr>
<tr>
<td>KR8500</td>
<td>SAMPLEBUF</td>
<td>Sample dilution buffer, ready-to-use</td>
<td>1 x 50 ml</td>
</tr>
<tr>
<td>KR0002.15</td>
<td>SUB</td>
<td>Substrate (tetramethylbenzidine), ready-to-use</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>KR0003.15</td>
<td>STOP</td>
<td>Stop solution, ready-to-use</td>
<td>1 x 15 ml</td>
</tr>
</tbody>
</table>

For reorders of single components, use the catalogue number followed by the label as product number.
4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Standard laboratory reaction vessels 1.5 ml (single-use)
- Standard laboratory reaction vessel (15 ml) (single-use)
- Foil to cover the microtiter plate
- Centrifuge, 3000 g
- Multi-channel pipets or repeater pipets
- Vortex
- Microtiter plate thermoshaker at 37 °C (for example model Shake ID2 available at Immundiagnostik AG)
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. Prepare only the appropriate amount necessary for each run. The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than 100 µl should be centrifuged before use to avoid loss of volume.

- Preparation of the wash buffer: The wash buffer concentrate (WASHBUF) has to be diluted with ultrapure water 1:5 before use (200 ml WASHBUF + 800 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redisolved at room temperature or in a water bath at 37 °C. The WASHBUF is stable at 2–8 °C until the expiry date stated on the label. Wash buffer (1:5 diluted WASHBUF) can be stored in a closed flask at 2–8 °C for 1 month.

Please note:

This WASHBUF is intended only for use in the IDK® DAO ELISA. Crystals in the WASHBUF must be completely dissolved before dilution.

- The lyophilised standards (STD) and controls (CTRL) are stable at 2–8 °C until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with 500 µl sample dilution buffer (SAMPLEBUF) and mixed by gentle inversion to ensure complete reconstitution. Allow the vial
content to dissolve for 10 minutes and then mix thoroughly. Standards and controls (reconstituted STD and CTRL) are not stable and cannot be stored.

- **Preparation of the conjugate and the detection antibody:** Before use, the conjugate concentrate (CONJ) and the detection antibody concentrate (AB) have to be diluted 1:101 in dilution buffer (100 µl CONJ + 10 ml ABBUF), (100 µl AB + 10 ml ABBUF). The CONJ and the AB are stable at 2–8 °C until the expiry date stated on the label. Conjugate (1:101 diluted CONJ) and detection antibody (1:101 diluted AB) are not stable and cannot be stored.

- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at 2–8 °C.

### 6. STORAGE AND PREPARATION OF SAMPLES

**Preanalytic handling**

Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.

**Serum**

**Sample storage**

The samples can be stored for 6 months at -20 °C. Avoid repeated freezing and thawing. The samples are stable at room temperature for up to 4 days and at 2-8 °C for up to 9 days.

**Sample preparation**

Serum samples must be diluted 1:5 before performing the assay, e.g. 50 µl sample + 200 µl sample dilution buffer (SAMPLEBUF), mix well. 100 µl of the dilution are used in the test per well.

**Dried blood spots**

**Collection and storage of dried blood spots**

50 µl whole blood dripped on a dried sample carrier cleared by Immundiagnostik AG are suitable as sample material after complete drying. We recommend DrySpot-ID (catalogue no DZ9020ID or DZ9021ID) as dried blood spot carrier.
**Preparation of the dried blood samples**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Label 1,5 ml polypropylene tubes.</td>
</tr>
<tr>
<td>2</td>
<td>Remove filter from sampling device (always wear gloves since the sample is potentially infectious).</td>
</tr>
<tr>
<td>3</td>
<td>Put filter in a labelled tube.</td>
</tr>
<tr>
<td>4</td>
<td>Add <strong>400 µl</strong> sample dilution buffer (SAMPLEBUF) per sample, allow sample to stand for <strong>20 min</strong> at room temperature (15–30 °C).</td>
</tr>
<tr>
<td>5</td>
<td>Vortex for <strong>10 sec.</strong> The filter will decolourise.</td>
</tr>
<tr>
<td>6</td>
<td>Centrifuge the samples for <strong>5 min</strong> at <strong>3000 g</strong> to remove residual filter pieces.</td>
</tr>
</tbody>
</table>

**7. ASSAY PROCEDURE**

**Principle of the test**

This ELISA is designed for the quantitative determination of DAO in serum and dried blood spots. The assay utilises the sandwich technique with two polyclonal antibodies against recombinant DAO.

Standards, controls and prepared samples which are assayed for DAO are added into the wells of a micro plate coated with polyclonal rabbit anti-DAO antibody. During the first incubation step, DAO is bound by the immobilised primary antibody. Then a biotinylated polyclonal anti-DAO antibody is added into each microtiter well. In the next step, the streptavidin peroxidase conjugate is added and a “sandwich” of 1st antibody – DAO - biotinylated antibody – streptavidin peroxidase conjugate is formed. Tetramethylbenzidine (TMB) is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of DAO. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. DAO, present in the samples, is determined directly from this curve.

**Test procedure**

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well. Mark the positions of standards/controls/samples on a protocol sheet.
Take as many microtiter strips as needed from kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Before use, wash the wells 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.</td>
</tr>
<tr>
<td>2.</td>
<td>Add each 100 µl standards/controls/prepared samples into the respective wells.</td>
</tr>
<tr>
<td>3.</td>
<td>Cover the strips and incubate for 2 hours at 37 °C on a horizontal shaker*.</td>
</tr>
<tr>
<td>4.</td>
<td>Discard the content of each well and wash 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.</td>
</tr>
<tr>
<td>5.</td>
<td>Add 100 µl detection antibody (diluted AB) into each well, mix gently.</td>
</tr>
<tr>
<td>6.</td>
<td>Cover the strips and incubate for 1 hour at 37 °C on a horizontal shaker*.</td>
</tr>
<tr>
<td>7.</td>
<td>Discard the contents of each well and wash 5 times with 250 µl wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.</td>
</tr>
<tr>
<td>8.</td>
<td>Add 100 µl conjugate (diluted CONJ) into each well.</td>
</tr>
<tr>
<td>9.</td>
<td>Cover the strips and incubate for 1 hour at 37 °C on a horizontal shaker*.</td>
</tr>
<tr>
<td>10.</td>
<td>Discard the contents of each well and wash 5 times with 250 µl wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.</td>
</tr>
<tr>
<td>11.</td>
<td>Add 100 µl substrate (SUB) into each well.</td>
</tr>
<tr>
<td>12.</td>
<td>Incubate for 10–20 min** at room temperature (15–30 °C) in the dark.</td>
</tr>
<tr>
<td>13.</td>
<td>Add 100 µl stop solution (STOP) into each well and mix well.</td>
</tr>
</tbody>
</table>
14. **Determine absorption immediately** with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

* We recommend shaking the strips at 700 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

### RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the “4 parameter algorithm”.

1. **4 parameter algorithm**
   
   It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. **Point-to-point calculation**
   
   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. **Spline algorithm**
   
   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

**Serum**

The obtained results have to be multiplied by the **dilution factor of 5** to get the actual concentrations.

**Dried blood spots**

The obtained results have to be multiplied by the **dilution factor of 6** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.
9. LIMITATIONS
Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.
Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.
The upper limit of the measurement range can be calculated as:
\[ \text{highest concentration of the standard curve} \times \text{sample dilution factor to be used} \]
The lower limit of the measurement range can be calculated as:
\[ \text{LoB} \times \text{sample dilution factor to be used} \]
LoB see chapter “Performance Characteristics”.

10. QUALITY CONTROL
Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.
Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range
We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n=22
The repeatability was assessed with 2 serum samples under constant parameters (same operator, measurement system, day and kit lot).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value [U/ml]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.98</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>3.84</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Reproducibility (Inter-Assay); n=20

The reproducibility was assessed with 3 serum samples under varying parameters (different operators, measurement systems, days and kit lots).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value [U/ml]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.98</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>11.26</td>
<td>8.9</td>
</tr>
<tr>
<td>3</td>
<td>23.58</td>
<td>8.7</td>
</tr>
</tbody>
</table>

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB 0.067 U/ml

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
• Substrate solution should remain colourless until use.
• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
• Avoid foaming when mixing reagents.
• Do not mix plugs and caps from different reagents.
• The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

• The guidelines for laboratories should be followed.
• IDK® is a trademark of Immundiagnostik AG.

• Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

• Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES


