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Manual

IDK® DAO ELISA

For the in vitro determination of DAO in serum

Valid from 2022-04-27

REF **K 8510**

Σ 96

+8°C +2°C

IVD CE

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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. For *in vitro* diagnostic use only.

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. In the case of DAO deficiency or inhibition, incorporated or endogenous histamine cannot be degraded quickly enough, and the symptoms of histamine intolerance are presented. Millions of people suffer from gastrointestinal problems, migraine, irritations of nasal mucosa and other allergy-like symptoms after consumption of certain nutrients. Too much histamine in the body can be the reason for this wide range of symptoms.

The determination of DAO serum concentration (K 8510) combined with the determination of DAO activity (K 8220 DAO REA) is a suitable marker for the differential diagnosis of histamine intolerance and associated symptoms.

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

Indications

- Frequent headaches or migraine
- Snuffles after consumption of histamine-containing nutrients
- Tissue oedema
- Eyelid turgor
- Skin redness
- Limb aches
- Gastrointestinal discomfort
- Monitoring of a histamine free diet

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 8510	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml

Cat. No.	Label	Kit components	Quantity
K 8510	CAL	Calibrator, lyophilised (see specification for concentration)	4 x 1 vials
K 8510	CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial
K 8510	CTRL2	Control, lyophilised (see specification for range)	4 x 1 vial
K 8510	AB	Detection antibody concentrate , lyophilised (biotinylated)	1 x 200 µl
K 8510	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 200 µl
K 8510	ABBUF	Dilution buffer for AB and CONJ, ready-to-use	1 x 50 ml
K 8510	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 50 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

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 10–1 000 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, 3 000 *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 **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The lyophilised **CAL** (calibrator) and **CTRL** (controls) are stable at 2–8 °C until the expiry date stated on the label. Before use, the CAL 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. **Calibrator and controls** (reconstituted CAL and CTRL) **are not stable and cannot be stored.**
- Use **100 µl SAMPLEBUF** (sample dilution buffer) as **BLANK**.
- **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. SAMPLE STORAGE AND PREPARATION

Preanalytic handling

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

Sample stability

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.

7. ASSAY PROCEDURE

Principle of the test

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

Calibrator, controls and diluted 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-POD-conjugate is added and a "sandwich" of

1st antibody – DAO - biotinylated antibody – streptavidin-POD-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. The concentration of DAO can be quantified by referring the optical density of the calibrator to a lot-dependendent master calibration curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of calibrator/controls/blank/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.

1.	**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.
2.	Add each **100 µl calibrator/controls/blank/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **2 hours** at **37 °C** on a **horizontal shaker***.
4.	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.
5.	Add **100 µl detection antibody** (diluted AB) into each well, mix gently.
6.	Cover the strips and incubate for **1 hour** at **37 °C** on a **horizontal shaker***.
7.	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.
8.	Add **100 µl conjugate** (diluted CONJ) into each well.
9.	Cover the strips and incubate for **1 hour** at **37 °C** on a **horizontal shaker***.
10.	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.
11.	Add **100 µl substrate** (SUB) into each well.
12.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.

13.	Add **100 µl stop solution** (STOP) into each well and mix well.
14.	Determine **absorption** immediately with an ELISA reader at **450 nm**. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used.

* 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.

8. RESULTS

For result evaluation, please use a four parametric logit-log model based on the standard curve of the respective kit lot and the calibrator value (CAL). All essential information on the standard curve is provided on the QC data sheet of the respective product lot.

The calibration curve can be expressed either by the concentration of each standard with its corresponding optical density or by the four parameters A,B,C and D. In both cases the optical density of the calibrator (CAL) is essential. Depending on your evaluation software program, either the one or the other kind of data described above should be entered.

Caution: Please make sure that all parameters and values are transferred accurately into your software as minor deviations can cause severe errors during evaluation.

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.

Samples

The obtained results have to be multiplied by the **dilution factor of 5** 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:

highest concentration of the calibration curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × 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 patient 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

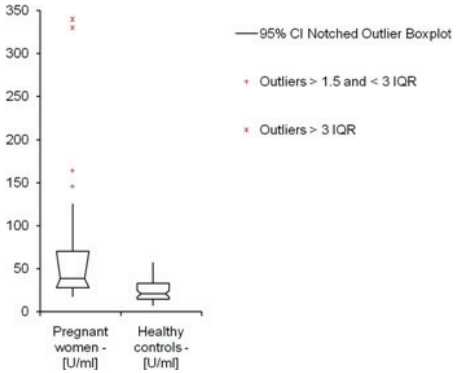
< 3 U/ml:	high incidence for HIT (Histamine intolerance)
3 – 10 U/ml:	HIT probable
> 10 U/ml:	low incidence for HIT

Conversion factor: 1 U/ml = 1.25 ng/ml

We recommend each laboratory to establish its own reference range.

Comparison "Pregnant women" and "Healthy controls"

For the clinical evaluation of this assay we have analysed samples from pregnant women and apparently healthy controls. The *IDK*® DAO ELISA detects, as required and expected, higher values in pregnant women than in healthy controls.



Heparin treatment

Furthermore, DAO levels in healthy study participants increased sharply within 30 minutes of heparin administration. It has been documented in scientific literature that DAO levels rise after heparin administration.

Treatment outcome before and after heparin administration

IDK® DAO ELISA [U/ml]

	BEFORE administration	30 min AFTER	60 min AFTER
Patient 1	76	219	-
Patient 2	55	152	-
Patient 3	2.5	277	622
Patient 4	18.9	621	555

Since the *IDK*® DAO ELISA determines DAO concentration while the conventional histamine intolerance tests with putrescine or histamine as substrates determine DAO activity, the correlation coefficient must not necessarily be r>0.8. This can be explained by the fact that the activity does not depend on the number of molecules alone, but also on cofactors such as vitamin C, vitamin B6, copper or manganese ions in vitro and in vivo. For the diagnosis of histamine intolerance via DAO activity test we therefore recommend to determine the above mentioned cofactors as well. The problem may not be a low DAO level, but a cofactor deficiency.

The symptoms of histamine intolerance can be caused by low DAO activity because the above-mentioned cofactors are not sufficiently available. By quantitating the cofactors it can be determined which one needs to be supplemented.

Medication effects

In addition, histamine intolerance symptoms may be due to low DAO activity caused by medication such as:

Muscle relaxants	Pancuronium, alcuronium, D-tubocurarine
Narcotics	Thiopental
Analgetics	Morphine, pethidine, nonsteroidal anti-inflammatory drugs, acetylsalicylic acid, metamizole
Local anesthetics	Prilocaine
Antihypotonics	Dobutamine
Antihypertensive drugs	Verapamil, alprenolol, dihydralazine
Antiarrhythmics	Propafenone
Diuretics	Amiloride
Drugs influencing gut motility	Metoclopramide
Antibiotics	Cefuroxime, cefotiam, isoniazid, pentamidin, clavulanic acid, choroquine
Mucolytics	Acetylcysteine, ambroxol
Broncholytics	Aminophylline
H2-receptor antagonists	Cimetidine
Cytostatics	Cyclophosphamide
Antidepressants	Amitriptyline

If you are taking such medication, you may want to discuss with your physician alternative medication in order to relieve your symptoms.

11. PERFORMANCE CHARACTERISTICS

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
Limit of detection, LoD	0.130 U/ml
Limit of quantitation, LoQ	0.195 U/ml

The evaluation was performed according to the CLSI guideline EP-17-A2. The specified accuracy goal for the LoQ was 20 % CV.

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, DAO-spikes with known concentrations were added to 4 different serum samples. The samples were diluted by the volume of the spike. This was considered when calculating the expected values.

Sample [U/ml]	Spike [U/ml]	Expected [U/ml]	Obtained [U/ml]	Recovery [%]
4.72	5.0	9.24	9.93	107.41
	2.5	6.98	6.89	98.64
	1.5	6.07	5.93	97.69
12.75	5.0	16.47	16.91	102.67
	2.5	14.61	14.21	97.24
	1.5	13.87	13.14	94.79
5.10	5.0	9.59	8.54	89.02
	2.5	7.34	6.59	89.75
	1.5	6.45	6.06	93.98
6.59	5.0	10.93	11.76	107.60
	2.5	8.76	8.89	101.52
	1.5	7.89	7.90	100.18

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline

EP06-A with a serial dilution of 5 different serum samples.

For DAO in serum, the method has been demonstrated to be linear from 0.41 to 9.18 U/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [U/ml]	Obtained [U/ml]	Recovery [%]
1	1:5	9.18	9.18	100.00
	1:10	4.59	4.63	100.82
	1:20	2.29	2.29	100.04
	1:40	1.15	1.18	102.66
2	1:5	3.30	3.30	100.00
	1:10	1.65	1.72	104.52
	1:20	0.82	0.88	106.58
	1:40	0.41	0.45	110.22
3	1:5	6.35	6.35	100.00
	1:10	3.17	3.14	98.90
	1:20	1.59	1.88	118.59
	1:40	0.79	0.94	118.82
4	1:10	5.78	5.76	99.57
	1:20	2.89	3.30	113.99
	1:40	1.45	1.79	123.58
5	1:10	6.92	6.82	98.57
	1:20	3.46	3.96	114.38
	1:40	1.73	2.09	120.98

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).

Sample	Mean value [U/ml]	CV [%]
1	19.98	2.2
2	3.84	5.0

Reproducibility (Inter-Assay); n = 20

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

Sample	Mean value [U/ml]	CV [%]
1	2.98	9.0
2	11.26	8.9
3	23.58	8.7

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic 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 or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10 x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

  Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.

- 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

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical 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

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Used symbols:


Temperature limitation

REF Catalogue number

IVD In Vitro Diagnostic Medical Device

→REF To be used with


Manufacturer

Contains sufficient for <n> tests

LOT Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet


Irritant