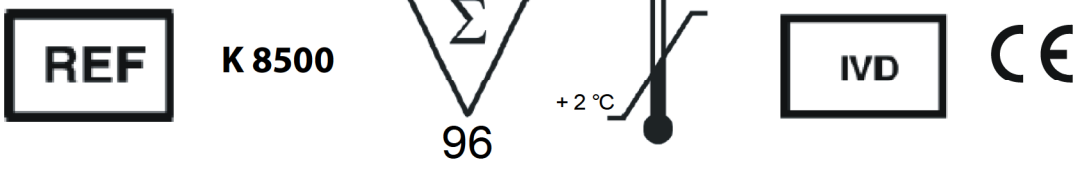
Manual

DAO ELISA

For the in vitro determination of DAO in serum and plasma

Valid from 8.3.2011



1. INTENDED USE

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is intended for the quantitative determination of diamine oxidase (DAO) in serum and plasma.

2. INTRODUCTION

Diamine oxidase (DAO) is a body's own enzyme that metabolizes 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.

Determination of the DAO concentration in serum and plasma is a suitable marker for diagnosis of histamine intolerance and associated symptoms.

Our DAO-ELISA kit is intended for determination of the damine oxidase (DAO) concentration in serum and plasma.

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	Content	Kit Components	Quantity
K 8500MTP	PLATE	One holder with precoated strips	12 x 8 wells
K 8500WP	WASHBUF	ELISA wash buffer concentrate 5x	4 x 100 ml
K 8500ST	STD	Standards, lyophilized (see specification for concentration range)	4 x 7 vials
K 8500KO1	CTRL	Control, lyophilized (see specification for concentration range)	4 x 1 vial
K 8500KO2	CTRL	Control, lyophilized (see specification for concentration range)	4 x 1 vial
K 8500A2	AB	Detection antibody, (biotinylated), concentrate	1 x 200 µl
K 8500K	CONJ	Conjugate (Streptavidin, peroxidase-labeled), concentrate	1 x 200 µl
K 8500VP	ABBUF	Dilution buffer for AB und CONJ, ready to use	1 x 50 ml
K 8500SV	STDBUF	Standard dilution buffer, ready to use	1 x 25 ml
K 8500PV	SAMPLEBUF	Sample dilution buffer, ready to use	1 x 50 ml
K 8500TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	1 x 15 ml
K 8500AC	STOP	ELISA stop solution, ready to use	1 x 15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Standard laboratory reaction vessels (1.5 ml)
- Standard laboratory reaction vessel (15 ml)
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Vortex mixer
- Ultra pure water*
- Shaking incubator at 37°C
- Microtiter plate reader at 450 nm

*Immundiagnostik AG recommends the use of Ultra Pure 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 0,055µS/cm at 25°C (≤18,2MΩ cm).

5. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. 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.
- The **WASHBUF** (wash buffer concentrate) should be diluted with ultra pure water **1:5** before use (200 ml WASHBUF + 800 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C in a water bath before dilution. **Mix well the diluted wash buffer before each use.** The **WASHBUF** (wash buffer concentrate) is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.

  Please note:
  This WASHBUF is intended only for use in the DAO-ELISA.
  Crystals in the WASHBUF must be completely dissolved before dilution.
- The lyophilized **STD** (standards) and **CTRL** (controls) are stable at 2-8°C until the expiry date stated on the label. The **STD** (standards) and **CTRL** (controls) must be reconstituted with **500 µl STDBUF** (standard dilution buffer). Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted standards are **not stable**.
- The **AB** (detection antibody, biotinylated) and the **CONJ** (conjugate, POD-antibody) must be diluted **1:101** in **ABBUF** (e.g. 100 µl AB + 10 ml ABBUF). The undiluted AB and CONJ are stable at **2-8 °C** until the expiry date given on the label. Diluted antibody and conjugate 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 of test package) when stored at **2-8°C**.

6. SAMPLE PREPARATION

Serum / Plasma

Pipet **25 µl** of fresh sample in a 1,5 ml reaction vial, add **225 µl SAMPLEBUF** (sample dilution buffer) and mix well (corresponds to **1:10 dilution**).

7. ASSAY PROCEDURE

Principle of the test

The assay utilizes the "sandwich" technique with two polyclonal antibodies against recombinant DAO.

Standards, 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 immobilized 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 color is directly proportional to the concentration of DAO. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. standard concentration is generated, using the values obtained from the standard.

Test procedure

1.	Prior to use in the assay allow all reagents and samples to come to room temperature and mix by gentle swirling and inversion.
2.	Mark the positions of **STD** (Standards)/**SAMPLE**/**CTRL** (Controls) in duplicate on a protocol sheet
3.	Take **PLATE** (microtiter strips) out of the kit. Store unused strips in the original package bag at 2-8° C. Strips are stable until expiry date stated on the label
4.	Wash the wells **5x with 250 µl** of diluted wash buffer. After the last wash, remove remaining wash buffer by hitting the plate against paper towel
5.	Add **100 µl of STD** (Standards)/**SAMPLE**/**CTRL** (Controls) in duplicate into respective well
6.	Cover the plate tightly and incubate for **2 hours at 37° C** on a horizontal mixer
7.	Aspirate and wash the wells **5x with 250 µl** of diluted wash buffer. After the last wash, remove remaining wash buffer by hitting the plate against paper towel
8.	Add **100 µl of AB** (Detection antibody, 2nd Antibody) into each wells mix gently
9.	Cover the plate tightly and **incubate for 1 hour at 37° C** on a horizontal mixer

10.	Aspirate and wash the wells **5x with 250 µl** of diluted wash buffer. After the last wash, remove remaining wash buffer by hitting the plate against paper towel
11.	Add **100 µl of CONJ** (Conjugate) into each well
12.	Cover the plate tightly and incubate for **1 hour at 37° C** on a horizontal mixer
13.	Aspirate and wash the wells **5x with 250 µl** of diluted wash buffer. After the last wash, remove remaining wash buffer by hitting the plate against paper towel
14.	Add **100 µl of SUB** (Substrate) into each well
15.	Incubate for **10 - 20 minutes at room temperature** (18-26°C) in the dark
16.	Add **50 µl of STOP** (Stop solution) into each well, shake well
17.	Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards **(STD)** is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. 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

*The intensity of the colour change is temperature sensitive. We recommend to observe the colour change and to stop the reaction upon good differentiation.

8. 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 calibrator must be specified with a value less than 1 (e. g. 0.01).

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 logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

   The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Samples

The obtained DAO concentration must be multiplied by a factor of **10**.

9. LIMITATIONS

Samples with DAO concentrations **outside the standard curve** range should be diluted further with **SAMPLEBUF** (sample dilution buffer) to obtain readings within the standard curve and re-assayed.

10. QUALITY CONTROL

Control samples should be analyzed 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.

Expected values

Normal range

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

Normal concentration range: Jarisch R et al. (1999)

It is recommended for each laboratory to establish its own normal range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

The precision (intra-assay variation) was calculated from 16 replicate determinations on each of two samples.

Intra-Assay CV n= 16

Sample	DAO [U/ml]	Intra-Assay CV [%]
1	5.0	1.42
2	5.9	1.72

The total precision (inter-assay variation) was calculated from data on 2 samples obtained in 8 different assays by different technicians on three different days.

Inter-Assay CV n= 8

Sample	DAO [U/ml]	Inter-Assay CV [%]
1	23.27	7.9
2	13.36	10.7

Sensitivity

The sensitivity limit was set as B_0 + 3 SD. The Zero-standard was measured 20 times.

Sensitivity n=20

Sample	Mean value [OD]	Standard variation	Detection limit [U/ml]
1	0.019	0.0036	0.52

Sample dilution

Two patient samples were diluted and assayed. The results are shown below:

Linearity n= 2

Sample	Dilution	Expected [U/ml]	Measured [U/ml]
A	undiluted	15,65	15,65
	1:10	7,83	7,68
	1:20	3,92	3,86
	1:40	1,96	1,86
B	undiluted	5,58	5,58
	1:10	2,79	2,89
	1:20	1,39	1,45
	1:40	0,7	0,7

12. PRECAUTIONS

- The quality control guidelines should be followed.
- Human material used in the kit components was 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.
- Reagents of the kit package contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulphuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.

13. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC
- The guidelines for medical laboratories should be followed.
- 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 wrong use.

- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG together with a written complaint.

15. REFERENCES

1. Sattler J et al. (1988) Food induced histaminosis as an epidemiological problem: plasma histamine elevation and haemodynamic alterations after oral histamine administration and blockade of diamine oxidase (DAO). Agents and Actions 23:361-65.
2. Tufvesson G et al. (1969) Determination of DAO-activity in normal human blood serum. Scand J Cli Lab Invest 24:163-68.
3. Wantke F et al. (1999) The red wine maximization test: drinking histamine rich wine induces a transient increase of plasma diamine oxidase activity in healthy volunteers. Inflammation Research 48:169-70.
4. Wantke F et al. (1994) The red wine provocation test: intolerance to histamine as a model für food intolerance. Allergy Proceedings 15:27-32.
5. Wantke F et al. (1998) Dailv variations of serum dliamine oxidase and the influence of H1 and H2 blockers: a critical approach to routine diamine oxidase assessment. Inflammation Research 47:396-400.
6. Jarisch R et al. (1999) Role of food allergy and food intolerance in recurrent urticaria. In: Wüthrich B (Hrsg): The Atopy Syndrome in the Third Millenium. Curr Probl Dermatol, Basel, Karger, 28:64-73.
7. Wantke F et al. (1993) Histamine free diet: treatment of choice for hiastamine induced food intolerance and supporting treatment for chronical headaches Clin Exp Allergy 23: 982-85.
8. Götz M et al. (1996) Histamin-Intoleranz und Diaminooxidasemangel Allergologie 9: 426-30.
9. Jarisch R (1999) Histamin Intoleranz. Stuttgart, Thieme

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

Contains sufficient for <n> tests


Manufacturer


Use by

Lot number