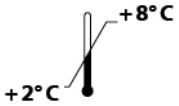
Manual

DAO-REA (^{3}H)

Radioextractionassay for the quantitative determination of diamine oxidase activity in serum

Valid from 14.10.2011

REF K 8220



RUO

Immundiagnostik AG

CE

Content

1. INTENDED USE

The described Radioextractionassay (REA) is intended for the quantitative determination of diamine oxicdase activity in serum and plasma. It is for research use only.

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. Another possibility for reduced DAO function could be the intake of activity-inhibiting substances, such as alcohol or medication.

Histamine induced food intolerance is not IgE-mediated. Determination of the DAO activity in serum or plasma is a suitable marker for diagnosis of histamine intolerance and the associated symptoms.

With our easy-to-use, reliable and standardised test kit it is possible to quantify the biological activity of DAO in the circulation. Only 100µl of serum is needed for the test, results are available within 3 hours.

Indications:

- Detection of histamine intolerace
- Monitoring of a histamine-free diet

3. MATERIAL SUPPLIED

Catalogue No.	Content	Kit Components	Quantity
K8220ST	STD	Standards with diamine oxidase from porcine kidney, lyophilized.	6 x1 ml
K8220KO1	CTRL	Diamine oxidase from porcine kidney, lyophilized	1 ml
K8220KO2	CTRL	Diamine oxidase from porcine kidney, lyophilized	1 ml
K8220AP	ASYBUF	assay buffer, ready to use	15 ml
K8220SUB	SUB	radiolabelled putrescine-dihydro-chloride in a stabilised solution., ready to use	5,5 ml
K8220EXT	EXTSOL	non-toxic, chlorine free extraction solution, ready to use	105 ml
K8220SZI	SZIN	Aquasafe Liquid Szintillation cocktail, ready to use	105 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Polypropylene-tubes with cap, holding 2 ml
- Pipettes for 50 µl, 100 µl, 800 µl, 1000 µl
- Multichannel or multipette
- Incubator (shaker) 37°C
- Vortex-mix
- Centrifuge
- Liquid szintillation vials
- Beta-Counter
- Software for calculation of results

5. STORAGE OF REAGENTS

- Freshly collected samples may be stored at room temperature or at 2-8°C up to 24 hours. For prolonged storage samples must be frozen at –20°C.
- Avoid more than 3 freeze cycles.
- The reconstituted standards (STD) and the controls (CTRL) are stable for 4 weeks at 2-8°C.
- All regents are stable at 2-8° C until the expiry date stated on the label

6. ASSAY PROCEDURE

Principle of the test

Human serum may be used as an analyte. The activity of the diamine oxidase is determined by quantitating the reaction product. Radiolabelled putrescine-dihydrochloride is used as a substrate. The resulting $\triangle^1$ pyrroline, containing the radiolabel, is extracted selectively from the matrix by a liquid extraction step. A non-toxic, chlorine free solvent with high capacity is used for the extraction.

Finally szintillation fluid is added to the organic phase containing the radiolabelled $\triangle^1$ pyrroline and radioactivity is determined in a beta-counter. The signal is directly proportional to the activity of DAO in the sample.

Procedural notes

- Samples should be mixed well before assaying.
- We recommend duplicates for all values.

Preparation

- The lyophilized standards (STD) and the controls (CTRL) must each be reconstituted with 1ml distilled H_2O. Allow the vial content to dissolve for 10 minutes; afterwards vortex well.
- All reagents should have reached room temperature (18°C to 26°C) before use. Prepare and label PP-tubes for standards, control and samples as appropriate.

Test procedure

Pipette 100 µl of reconstituted standards (STD), controls (CTRL) and samples into the respective tubes
Add 100 µl of assay buffer (ASYBUF) into all tubes
Add 50 µl of substrate (SUB) to all tubes
Vortex all tubes thoroughly
Incubate all tubes for 150 min at 37°C, shake if possible
Add 1 ml of extraction solution (EXTSOL) into all tubes, mix thoroughly on a vortex mixer for at least 15 seconds This step is essential for a complete extraction!
Centrifuge the extraction mixture for 1 min at > 3000 x g
Transfer 800 µl of the upper phase (organic) into a szintillation vial **(Alternative method of running the test, see below *)**
Add 1 ml of szintillaton (SZIN) fluid into all szinti-vials, mix well
Count every vial for 1 min in the beta counter

***Alternative method of running the test:**
<u>Decantation of the supernatant:</u> Alternatively the reaction tubes can be incubated at -20°C for at least 90 minutes to freeze the lower (red) phase. The organic supernatant remains liquid and can easily be decanted directly into the scintillation vials. It's necessary that the entire lower phase is frozen to make sure that it completely remains in the tube while decanting the organic phase. Please avoid the usage of polystyrene tube racks or increase the incubation time at -20°C. Metal or plastic tube racks are recommended. A preincubation of these racks at -20°C can reduce the required incubation time. In any case it is highly recommended to make sure that a constant phase separation is warranted.
Transfer 800 µl of the upper phase (organic) into a szintillation vial
Add 1 ml of szintillaton (SZIN) fluid into all szinti-vials, mix well
Count every vial for 1 min in the beta counter

7. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use 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 has to be specified with a value smaller 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 for the optical density a linear ordinate and for the concentration a logarithmic abscissa. When using a logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e. g. 0.01).

8. QUALITY CONTROL

Immundiagnostik AG recommends the use of commercial control samples for internal quality control if available.

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

< 3 U/ml:	high incidence for HIT
3 - 10 U/ml:	HIT probable
> 10 U/ml:	low incidence for HIT

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

We recommend each laboratory to establish its own norm concentration range.

A diagnosis of histamine-intolerance must be correlated to the actual concentration of histamine.
It is IMPOSSIBLE to state histamine intolerance in case of anaphylactic shock and during pregnancy!

9. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n=10)		
Sample	DAO-Activity (U/ml)	VK [%]
1	41,5	4,07
2	2,8	6,29
3	27,5	4,78

Inter-Assay (n=5)		
Sample	DAO-Activity (U/ml)	VK [%]
1	42,4	5,59
2	2,7	16,16
3	27,4	4,39

Sensitivity

The sensitivity was set as 0,2 U/ml (B0 + 3 SD). The zero-standard was measured 10 times.

Recovery:

2 serums were spiked with 10 U/ml DAO and measured with the assay.

Serum	Unspiked	Expected	Measured	Recovery
1	19,1	29,1 U/ml	29,7 U / ml	102,1 %
2	1,5	11,5 U/ml	9,6 U / ml	83,5 %

10. PRECAUTIONS

- For in research use only.
- The quality control guidelines should be followed.
- Avoid all contact with the reagents by using gloves.
- Radioactive waste must be disposed according to local regulations.
- The extraction solvent is a non-halogenated organic solvent and has to be disposed according to local regulations.

11. TECHNICAL HINTS

- Extraction time must not be shorter than 15 sec as extraction recovery may vary with shorter extraction times.
- Do not use reagents beyond expiration date.
- Do not expose reagents to direct sunlight.
- Do not mix or substitute reagents with those from other lots or sources.
- The assay should always be performed according to the enclosed manual.

12. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- 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 coordina-ted 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 shall be send to Immundiagnostik AG together with a written complaint.

13. REFERENCES

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Used Symbols:


Temperature limitation

REF Catalogue Number

RUO For Research Use Only


Contains sufficient for <n> tests


Manufacturer


Radioactivity