ALPHA DIAGNOSTIC INTERNATIONAL

Product Data Sheet

D-Amino acid oxidase

□ **Cat#** DOX-01 D-Amino acid oxidase (6000 U/g), Porcine Kidney **Size**: 1 KU

D-amino acid oxidase (DAAO; also DAO, OXDA, DAMOX) is a peroxisomal enzyme containing FAD as cofactor that is expressed in a wide range of species from yeasts to human. It is not present in plants or in bacteria which instead use D-amino acid dehydrogenase. Its function is to oxidize D-amino acids to the corresponding imino acids, producing ammonia and hydrogen peroxide. This enzyme belongs to the FAD dependent oxidoreductase family, and acts on the CH-NH2 group of D-amino acid donors with oxygen as acceptor. The enzyme is most active toward neutral D-amino acids, and not active toward acidic D-amino acids.

Recently, mammalian D-amino acid oxidase has been connected to the brain D-serine metabolism and to the regulation of the glutamatergic neurotransmission. In a postmortem study, the activity of DAAO was found to be two-fold higher in schizophrenia. DAAO is a candidate susceptibility gene and together with G72 may play a role in the glutamatergic mechanisms of schizophrenia.[4] Risperidone is an inhibitor of DAAO. DAAO is used as a biocatalyst in several biotechnological applications, such as the oxidation of cephalosporin C, the deracemition of racemic D-amino acid solutions and as the biological component in several biosensors for the determination of the content in D-amino acids of biological fluids.

Human D-AAO is 347-aa. Pig DAAO 347-aa.
E.C. number: 1.4.3.3
CAS number: 9000-88-8
EINECS number: 232-563-5
Systematic name: D-Amino-acid:oxygen oxidoreductase (deaminating).

Source: Porcine kidney

Form: A freeze-dried material.

Storage conditions: Store desiccated at -15 C or below. Allow to come to room temperature before opening. Before returning to storage, redesiccate under vacuum over silica gel for a minimum of four hours.

Re-seal before returning to -15 C or below.

Unit definition: That amount of enzyme causing the transformation of one micromole of D-alanine to pyruvate per minute at 25 C and pH 8.3 in the presence of catalase.

Activity: Not less than **6,000 U/g material** (not less than 7,000 U/g protein).

This material is fully active without the addition of FAD.

Solubility: Dissolves readily at 5mg/ml in analytical grade water to give a clear solution.

References: Pollegioni L (2007) Cell Mol. Life. Sci. 64, 1373-1394; Maderia CD (2008) Schizoph. Res. 101, 76-83; Boks MP (2007) Eur Neuropsychopharmacol 17 (9): 567–72.

Related Material available for ADI

DOX-01 140723A

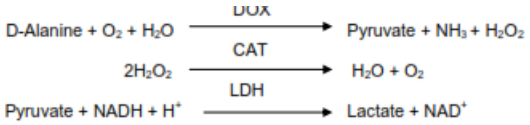
Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com

D- Amino acid oxidase Assay

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: D-Amino acid oxidase
1.2 **Systematic Name**: D-Amino acid: oxygen reductase (deaminating)
1.3 **E.C. Number**: 1.4.3.3
1.4 **Source**: Porcine kidney
1.5 **Suitable for BBI Solutions codes**: DOX2, DOX3S

2.0 ASSAY PRINCIPLE



3.0 UNIT DEFINITION

That amount of enzyme causing the transformation of one micromole of D-Alanine to pyruvate per minute at 25 C and pH 8.3, in the presence of catalase.

4.0 EQUIPMENT REQUIRED

Double beam UV/vis recording spectrophotometer, with a cell compartment thermostatically controlled at 25 C (0.1 C).
Water bath set at 25 C (0.5 C).
Silica cuvettes, glass test tubes, glass pipettes and automatic pipettes.
Heated magnetic stirrer and magnetic follower.
pH meter readable to 0.01 pH unit.

5.0 REAGENTS REQUIRED

When using the following reagents please refer to the manufacturer's instructions for safe handling and disposal.

Reagent details

D-Alanine Hydrochloric acid AnalaR
Catalase Lactate dehydrogenase (from bovine heart)
Di-Potassium hydrogen orthophosphate trihydrate (K2HPO4.3H2O)
Nicotinamide adenine dinucleotide (reduced) disodium salt (anhydrous) (NADH-Na)
Oxygen
Potassium dihydrogen orthophosphate (KH2PO4) AnalaR
Tris(hydroxymethyl)methylamine (NH2.C (CH2.OH3)

6.0 PREPARATION OF REAGENTS

6.1 5.8M Hydrochloric acid. (Reagent 1)

Carefully add 100ml of concentrated Hydrochloric acid to 100ml of analytical grade water and stir for approximately 5 minutes. Stable at room temperature for 1 month.

6.2 0.2M Tris/HCl pH 8.3 (Reagent 2)

Dissolve 12.12g of Tris (hydroxymethyl) methylamine in approximately 450ml of analytical grade water. Warm to 25 C, adjust to pH 8.3 with 5.8M HCl (Reagent 1) then make up to 500ml with analytical grade water. Stable at 2-8 C for 1 week.

6.3 0.56M D-Alanine. (Reagent 3)

Dissolve 1g of D-Alanine in approximately 15ml of analytical grade water, then make up to 20ml with the same. Stable at 2-8 C for 5 days.

6.4 0.05M Potassium phosphate pH 7.4. (Reagent 4)

0.05M KH2PO4
Dissolve 0.340g of Potassium di-hydrogen orthophosphate in 40ml of analytical grade water, then make up to 50ml with the same.

0.05M K2HPO4
Dissolve 1.14g Dipotassium hydrogen orthophosphate trihydrate in 80ml of analytical grade water and make up to 100ml with the same.
Titrate the 0.05M K2HPO4 with the 0.05M KH2PO until a pH of 7.4 is obtained. Stable at 2-8oC for 2 weeks.

6.5 Buffered water (0.005M potassium phosphate pH 7.4). (Reagent 5)
Add 10ml of 0.05M Potassium phosphate, pH 7.4, (reagent 4) to 90ml of analytical grade water. Cool until a thin film of ice forms on the inside of the reagent bottle. Prepare fresh daily.

6.6 0.008M NADH solution. (Reagent 6)
Dissolve 28.4mg of Nicotinamide adenine dinucleotide (reduced) disodium salt (anhydrous)in 5ml of ice-cold buffered water (Reagent 5). Stable at 2-8 C for 5 days.

6.7 **Lactate dehydrogenase solution** (Reagent 7)
Dilute Lactate dehydrogenase to a concentration of 250U/ml in ice-cold 0.2M Tris/HCl, pH8.3 (Reagent 2). Store on ice and prepare fresh daily.

6.8 **Catalase solution.** (Reagent 8)
Dissolve Catalase up to a concentration of 1mg/ml in ice-cold 0.2M Tris/HCl, pH 8.3
(Reagent 2). Dilute further to a concentration of 250U/ml in the same buffer). Store on ice and prepare fresh daily.

6.9 **Enzyme solution. Freeze-dried preparations.**
Accurately weigh approximately 10mg of enzyme and add analytical grade water to give a5mg/ml solution. Allow to stand at room temperature for approximately 5 minutes, swirl gently to dissolve, then store on ice. Immediately prior to measurement, dilute further to approximately 0.20U/ml in analytical grade water.

Ammonium sulphate suspensions
Immediately prior to measurement, dilute approximately 0.20 U/ml in analytical grade water.

7.0 TEST PROCEDURE

Temperature = 25 C Wavelength = 340nm Light path = 1cm

Sparge Reagent 2 (0.2M Tris/HCl pH 8.3) with oxygen for approximately 10 minutes before use. Replace at 2-8 C after use and resparge every 30 minutes.

Into test tubes at 25 C pipette the following:

	TEST	**REF**
Oxygenated 0.2M Tris/HCl pH 8.3 (Reagent 2)	2.50ml	2.60ml
0.56M D-Alanine (Reagent 3)	0.20ml	0.20ml
0.008M NADH solution (Reagent 6)	0.10ml	0.10ml
Lactate dehydrogenase (Reagent 7)	0.10ml	0.10ml
Catalase solution (Reagent 8)	0.10ml	0.10ml
Enzyme solution at approx. 0.2U/ml	0.10ml	0.00ml
	3.10ml	**3.10ml**

Transfer to matched silica cuvettes and record the decrease in absorbance at 340nm,reading the test solution versus the reference solution, for approximately 5 minutes.Measure the change of absorbance per minute over the linear portion of the curve and usethis value in the calculation.

8.0 CALCULATION

$$\text{Volume activity (U/ml)} = \frac{\Delta E_{340}/\text{min} \times Vt \times \text{dilution factor}}{Vs \times \varepsilon}$$

Where:

Vt = final volume in cuvette (mls) = 3.10
Vs = sample volume in cuvette (mls) = 0.10
ε = micromolar extinction coefficient for NADH = 6.22

Volume activity (U/ml) = ΔE_{340}/min x 4.98 x dilution factor

Specific activity (U/mg protein) = $\frac{\text{U/ml}}{\text{mg protein/ml}}$ (Ammonium sulphate suspensions)

Weight activity (U/mg material) = $\frac{\text{U/ml}}{\text{mg material / ml}}$ (Freeze-dried powders)

Specific activity (U/mg protein) = $\frac{\text{U/mg material}}{\text{mg protein/mg material}}$

9.0 PROTEIN DETERMINATION

Protein is determined by the method Lowry *et al2* (see Procedure No, AP62, Analytical Procedures Manual).

10.0 E1%/280 DETERMINATION

The E 1%/280 is determined according to Proc. No. AP63 (Analytical Procedures Manual).

11.0 REFERENCES

1. Bergmeyer *et al* (1983) *Methods of Enzymatic Analysis*, Verlag Chemie, vol. 2, page 149, (3rd edition).
2. O.H Lowry *et al*. J. Biol. Chem. (1951), **193**, 265.