

ARGINASE (EC 3. 5. 3. 1)

Tumour marker

Colorimetric Method

25 Test

INTRODUCTION :

Arginase (EC 3.5.3.1) is one of the essential enzymes in the terminal stages of the urea cycle in the liver which participates in the elimination of ammonia from the human body. Except in liver tissue arginase is also present in many human tissues and in the circulating blood cells, especially in erythrocytes and leukocytes. Arginase splits arginine to urea and ornithine that serve for biosynthesis of amino acid proline, glutamic acid and biosynthesis of polyamines-spermine, spermidine and putrescine.

Arginase activity is high during the mitotic cycle, with the function in phase S of the cell cycle. The measurement of arginase activity in plasma and erythrocytes is a good diagnostic indicator for the presence of young erythrocytes and reticulocytes in the circulating blood as is the good sign for the detection of haemolytic processes.

The polyamine metabolism in prostatic tissue, which may give rise to prostatic hyperplasia by inducing cell proliferation, is primarily by two enzyme: arginase and diamine oxidase (DAO). Arginase catalyzes the synthesis of ornithine the precursor for polyamines from arginine, whereas DAO catalyzes the oxidation of diamines, such as spermine and spermidine, to a much less active compound called putrescine. Both DAO and arginase activities were found to be elevated in cancer tissues as compared to benign prostatic hyperplasia (fivefold and twofold, respectively; $P < .001$). DAO and arginase might play an essential role in the mechanism of prostatic disease process by modulating polyamine levels.

The mean serum arginase activity was increased in all liver diseases. As compared with serum from healthy blood donors the activity was 2-3 times higher in patients with hepatocellular carcinoma, 4-5 times higher in patients with liver cirrhosis and 20-30 times higher in patients with colorectal cancer liver metastases. Serum arginase activity can be helpful in the diagnosis of patients with colorectal cancer liver metastases.

Erythrocyte arginase activity is significantly increased in lung cancer patients, with no additional effect of cell type or extrapulmonary metastases.

PRINCIPLE :

A number of assay procedures have been used for arginase in tissue homogenates and erythrocyte.

The method used by Biodiagnostic for plasma and serum is based upon the colorimetric determination of urea by condensation with diacetyl monoxime in an acid medium in the presence of ferric chloride (oxidant) and carbazide (accelerator).

SAMPLE

Sera It is recommended to spin the erythrocytes down immediately (within few seconds) after taking blood.

It is not possible to get reliable results when measuring arginase in normal serum. Trace hemolysis and contamination of serum with erythrocyte arginase causes false increased results.

Samples should be stored frozen (preferably at -80°C , then the stability is at least 1 year).

Repeated thawing-freezing cycles should be avoided.

REAGENTS :

1.	Standard Urea	50 mg / dL 8.31 mmol / L
2.	Activator (Manganes Sulphate)	100 mmol / L
3.	Buffer-Substrate Carbonate buffer pH 9.5 Arginine	200 mmol / L 1.0 mol / L
4.	Acid Mixture Sulphuric acid Phosphoric acid	1.0 mol / L 4.0 mol / L
5.	Diacetyl monoxime (DAMO)	50 mmol / L

STABILITY :

The reagents are stable up to the expiry date specified when stored at $+2$ to $+8^{\circ}\text{C}$.

PROCEDURE :

	Sample (mL)	Sample Blank (mL)	Standard (mL)	Standard Blank (mL)
Sample	0.01	0.01	-	-
Standard (R1)	-	-	0.01	-
Activator (R2)	0.01	-	-	-
Buffer/ subst. (R3)	0.2	-	-	-
D. Water	-	0.2	0.2	0.2
Incubate for 60 min. exactly at 37°C , then add:				
Acid Mix (R4)	1.0	1.0	1.0	1.0
DAMO (R5)	0.5	0.5	0.5	0.5

Mix well. Place tubes, covered with glass bead, in boiling water bath for 10 min. Cool. Measure the absorbances of the sample (A_{Sample}) against sample blank and of the standard (A_{Standard}) against the standard blank at 525 nm. (490 – 530 nm) Color stable over night.

Linearity up to 250 U/L.

CALCULATION :

Arginase activity in Serum (U/L)

$$= \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times 138$$

REFERENCE :

Marsch, W. et al. (1965) Chin. Chem. 11, 624

ARGINASE

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+2 to +8°C

25 Tests

CAT. NO.

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FOR RESEARCH USE ONLY

REAGENTS

R1 Standard	2 ml
R2 Activator	3 ml
R3 Buffer- Substrate	10 ml
R4 Acid Mixture	50 ml
R5 DAMO	25 ml

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