

ALA - DEHYDRASE

Colorimetric determination
of Erythrocyte ALA – Dehydrase activity

50 tests

REF CM03-50T

INTENDED USE

Kit for quantitative *in vitro* determination of Erythrocyte Ala-Dehydrase on blood.

PRINCIPLE

ALA-dehydrase enzyme is extracted from erythrocytes and catalyzes the transformation of 5-aminolevulinic acid into porphobilinogen. The reaction product is directly proportional to the enzyme activity and it is defined by Ehrlich's reaction and the quantity is defined photometrically.

REAGENTS

Kit components:

REF CM03-50T

REAGENT 1 Hemolyzer solution	1 x 50 ml
REAGENT 2/A 5-aminolevulinic acid (powder)	2 vials
REAGENT 2/B Phosphate buffer	2 x 14 ml
*REAGENT 3 Trichloroacetic acid	1 x 80 ml
REAGENT 4/A p-Dimetilaminobenzaldehyde (powder)	1 vial
*REAGENT 4/B Acetic acid	1 x 100 ml
*REAGENT 4/C Perchloric acid	1 x 20 ml

(*) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 2-8°C, sealed reagents are stable to the expiration date on the label.

REQUIRED BUT NOT PROVIDED

Bain-marie, centrifuge, spectrophotometer or filter (520-570 nm).

PREPARATION OF WORKING REAGENTS

REAGENT 2 (2/A + 2/B)

Put the fracture cap of Reagent 2/A on the vial of Reagent 2/B; push to the bottom and shake until complete dissolution.

STABILITY: 2 months at 2-8°C.

REAGENT 4 (4/A + 4/B)

Dissolve the contents of the Reagent 4/A into the vial of Reagent 4/B and shake until complete dissolution.

STABILITY: 4 months at 2-8°C.

Thaw the solution at room temperature or in a bain-marie (20-30°C) away from direct light.

EHRlich REAGENT (4/A + 4/B + 4/C)

Add 1.9 ml of Reagent 4/C to 10 ml of Reagent 4. Shake to obtain a homogeneous solution. The solution thus prepared will be enough for 5 assays. If required, higher quantities can be prepared taking into consideration that each test needs 2 ml of this reagent.

STABILITY: **6 hours at room temperature.**

SAMPLE

Heparinized blood.

Store the sample at 2-8°C and perform the test as soon as possible.

STABILITY: store at 2-8°C. After 24 hours at 2-8°C, the enzyme activity decreases of approx. 4-10%.

MANUAL ASSAY PROCEDURE

Wavelength:	553 nm (520 – 570 nm)
Optical path:	1 cm
Reading:	against blank reagent
Temperature:	37°C
Method:	colorimetric end point
Linearity:	up to 10 U/ml
Sensitivity:	0.2 U/ml
C.V.:	1%

Carefully pipette into a centrifuge test tube:

Sample	0.1 ml
Reagent 1	0.9 ml

Mix thoroughly and add:

Reagent 2	0.5 ml
-----------	--------

Mix accurately, then incubate for 1 hour at 37°C away from light. Remove the testtubes from bain-marie and pipette into each one:

Reagent 3	1.5 ml
-----------	--------

Mix and centrifuge 5 minutes at 3000 rpm.

Pipette into dry test tubes:

	Blank reagent	Sample
Supernatant	---	1.5 ml
Distilled water	1.5 ml	---
Ehrlich Reagent	1.5 ml	1.5 ml

Mix thoroughly. After exactly 10 minutes, read the standard absorbance (As) against blank reagent.

CALCULATION

ALA-dehydrase activity (standardized European method):

Units (ALA-D)/ml erythrocytes = (Ac / Hematocrit %) x 3226

REFERENCE

Adults > 20 U/ml

Each laboratory should define its own reference values.

NOTES

- The enzymatic activity is expressed in units/ml (in accordance with the standardized European method), corresponding to the substrate nanomoles transformed per minute and per erythrocytes ml.
- Calibration of the photometer: dissolve 0.5 g of phenolphthalein in 100 ml of absolute ethylic alcohol and add 0.5 ml of this solution to 100 ml of buffer borate 0.01 mol/L pH 9.22. After resetting by borate in a cuvette of 1 cm optical path, a properly calibrated instrument should read absorbance A = 0.610, at $\lambda = 555$ nm. Otherwise, calculate the following k correction factor: $k = 0.610/A$ read where "A read" is the absorbance value actually measured. During calculation, multiply the result in U/ml by this correction factor.

REFERENCE

- A. Berlin et K.H. Schaler, "Z. Klein. Chem. Klein. Biochem." 12, 389-390 (1974).

KEY SYMBOLS

	In Vitro diagnostic medical device
	batch number
	catalogue number
	temperature limits
	use by
	caution
	read instructions for use

IVD

CE

Ed. 01 - Jan 2022

MANUFACTURER



FAR

Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY

phone +39 045 6700870

website <http://www.farddiag.com>

e-mail: order@farddiag.com e-mail: farddiag@farddiag.com