DATA SHEET

Adenosine Deaminase Assay Kit
Catalog Number: BQ 014 - EALD

Intended Use
Adenosine deaminase (ADA) assay kit is for the determination of ADA activity in human serum, plasma, pleural fluid and cerebrospinal fluid (C.S.F.) samples. For investigational use or export only.

Clinical Significance
ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, especially high in T lymphocytes. Elevated serum ADA activity has been observed in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, viral hepatitis and hepatoma.[1,2] Increased ADA activity was also observed in patients with tuberculous effusions.[3] Determination of ADA activity in patient serum may add unique values to the diagnosis of liver diseases in combination with ALT or γ-GT (GGT) tests. ADA assay may also be useful in the diagnostics of tuberculous pleuritis.[3]

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Assay Principle
The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N – Ethyl – N - (2-hydroxy-3-sulfopropyl) - 3 -methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Inosine} + NH_3$$

$$\text{Inosine} + Pi \xrightarrow{\text{PNP}} \text{Hypoxanthine} + \text{Ribose 1-phosphate}$$

$$\text{Hypoxanthine} + 2H_2O + 2O_2 \xrightarrow{\text{XOD}} \text{Uric acid} + 2H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye } (\lambda \text{ max } 556\text{nm})$$

One unit of ADA is defined as the amount of ADA that generates one µmole of inosine from adenosine per min at 37 °C.

Materials Required but not Provided
Any instrument with temperature control of 37 +/- 0.5 °C that is capable of reading absorbance accurately at 540 – 550 nm may be used.

Reagent Preparation
Liquid two-reagent system, ready-to-use for both manual method and automated chemistry analyzers (kinetics). ADA Control and calibrator are in lyophilized form, and need to be reconstituted with 1.0 mL of water before use. The reconstituted ADA control or calibrator is stable for 1 week at 4 °C. Control is sold separately.

Regent Stability and Storage
Reagents are stable until their expiration date when stored at 2 – 8

Reagent Composition (275 tests)

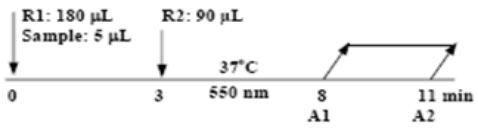
Active Ingredients	Concentration
Reagent 1 (R1) - 50 mL	
Tris HCl, pH 8.0	50 mM
4-AA	2 mM
PNP	0.1 U/mL
XOD	0.2 U/mL
Peroxidase	0.6 U/mL
Stablizers	
Reagent 2 (R2) - 25 mL	
Tris HCl, pH 4.0	50 mM
Adenosine	10 mM
EHSPT	2 mM
ADA Calibrator - 2.0 mL	

Sample Specimen Collection and Handling
Serum or heparinized plasma may be assayed. Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant.

Plasma and serum, after prompt separation from cells or clot, should be kept tightly stoppered. ADA content of blood is stable for one (1) week at 2-4 °C.

Assay Procedure

Test Scheme for Chemistry Analyzers



Calibration
A single calibrator, along with 0.9% saline as a zero reference, should be used as directed to calibrate the procedure.

Quality Control
Bio-Quant recommends that each laboratory use ADA controls to validate the performance of ADA reagents. An ADA control is available from Bio-Quant. If the results from the control falls outside the acceptable limits, which is +/- 15% from the target value, the test should not be performed. We recommend that your quality control testing follows federal, state, and local guidelines.

Results
The ADA results are printed out in U/L.

Reference Range
The ADA activities in 60 healthy human serum samples were found to be in the range of 0-15 U/L. For pleural fluid, values were found to be in the range of 0-24 U/L, and for C.S.F., values were found to be in the range of 0-5 U/L. It is recommended that each laboratory establish its own range of reference values.

Limitations
Assay is specific for ADA and has no detectable reaction with other nucleosides. The reagent solution should be clear. If turbid, the reagent may have deteriorated.
If the samples ADA activity is greater than 200 U/L, the sample should be diluted with saline before measurement. The result should be multiplied by the dilution factor.

Performance Characteristics
These performance characteristics were determined at Bio-Quant laboratories using automated procedures unless otherwise stated.

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Precision
The precision of the Bio-Quant ADA assay evaluated on the Cobas Mira instrument according to Clinical laboratory Standards Institute (formerly NCCLD) EP5-A guideline. In the study, two serum specimens containing 11 U/L and 30 U/L ADA were tested with 2 runs per day with duplicates over 15 working days.

	Within Run Precision		Run to Run Precision	
	11 U/L	30 U/L	11 U/L	30 U/L
No. of Data Points	30	30	30	30
Mean (µM)	11.1	30.6	10.57	29.9
SD (µM)	0.21	0.56	0.23	0.64
Cv %	1.4	1.8	2.13	2.13

Assay Linearity

The linearity of the procedure is from 0 - 200 U/L.

Interference
Assay is not affected by serum bilirubin up to 20mg/dL, hemoglobin up to 200mg/dL, triglycerides up to 750mg/dL, and ascorbic acid up to 4mg/dL.

Safety Precautions and Warnings

1. Reagent R1 is light-sensitive. Store in a dark place.
2. Specimens containing human sourced materials should be handles as if potentially infectious using safe laboratory procedures, such as those Biosafety in Microbiological and Biomedical Laboratories (HHS publication Number [CDC] 93-8395).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
5. The reagents contain <0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
6. Do not use the reagents after the expiration date labeled on the outer box.

References

1. Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. *Am. J. Gastroenterol.* 88: 266-271 (1993)
2. Kallkan A., Bult V., Erel O., Avci S., and Bingol N. K. : Adenosine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. *Mem Inst. Oswaldo Cruz* 94(3) 383-386 (1999)
3. Burgess LJ, Maritz FJ, Le Roux I, et al. Use of adenosine deaminase as a diagnositic tool for tuberculous pleurisy. *Thorax* 50: 672-674 (1995)

Cobas Mira-S Parameters
Temperature 37°C

Measurement Mode	Absorb
Reaction Mode	R-S-SR1
Calibration Mode	SLOPE AVG
Reagent Blank	Reag/DIL
Cleaner	No
Wavelength	550 nm
Decimal position	2
Unit	U/L
Sample Cycle	1
Sample Volume	5.0 uL
Sample dilution	H_20
Dilution volume	0.0 uL
Reagent cycle	1
Reagent volume	180 uL
Dilution volume	0.0 uL
Start R1 cycle	7
Reagent volume	90 uL
Dilution volume	0.0 uL
Sample limit	No
Reaction Direction	Increase
Convers. Factor	1.0000
Offset	0.0000
Test range Low	0.000 U/L
Test range High	200.00 U/L
Number of steps	1
Calc. Step A	Kinetics
Readings first	19
Readings last	27
Calibration	
Cali. Interval	Each day
Time	No

Blank		
Reagent Range	Low	-0.1
	High	0.3
Blank Range	Low	-0.1
	High	0.1
STANDARD	POS	1
	STD-1	*

* Entered by operator

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Hitachi 717 Parameters
Temperature 37°C

Test	ADA
Assay Code	Rate-A
Assay Point	(10) (27) (34)
Wavelength (Sub/Main)	(750) (546)
Calibration Type	Linearity
Sample volume (Normal)	(5) (0) (0)
Sample volume (Dec.)	(5) (0) (0)
Sample volume (Inc.)	(5) (0) (0)
Diluent	(water) (0)
Reagent vol. R1	(180) (0) (10008) (0)
Reagent vol. R2	(0) (0) (1000) (0)
Reagent vol. R3	(90) (10008) (0) (0)
Reagent vol. R4	(0) (0) (1000) (0)
ABS. Limit	(32000) (Increase)
STD. (1) CONC. – Position	(0)-(1)
STD. (1) CONC. – Pos	(*)-(2)
Expected value (normal value)	4-20
Tech. Limit	0-200

** Each cycle is 12 seconds.
* Entered by operator

Olympus AU400 Parameters
Calibration Method - Temperature 37°C

General

Test Name: 3. ADA Type: Serum Operation: Yes

Sample Volume 5.0 µL Dilution 0 µL Pr-Dilution Rate 1

Reagents : Min OD Max OD

R1 volume 180 µL Dilution 0 µL L:-2.000 H:2.500

R2 volume 90 µL Dilution 0 µL

Wavelength: Pri. 540 Sec. 700 Reagent OD Limit:

Method: Rate First L: -2.000; First H: 2.500

Reaction Slope: + Last L: -2.000; Last H: 2.500

Measuring Point 1: First 20; Last 27 Dynamic Range:

Measuring Point 2: First Last L:0.0 H:200.0

Linearity 20% Correlation Factor:

No-Lag-Time: No A:1.0000 B:0.000

Onboard stability Period: 999

Calibration Type AB Formula: Y=AX+B

Counts 2 Process CONC

Cal No. OD CONC Factor/OD-L Factor/OD-H

Point 1 *

Point 2

Advanced Calibration: No

MB Type Factor: Calibration Stability Period: 999

* Entered by operator

Beckman Synchron CX-7 Delta Parameters
Temperature 37°C

CHEMISTRY NAME: Adenosine Deaminase
TEST NAME: [ADA]

REACTON TYPE: RATE 2 MATH MODEL: LINEAR
REACTION DIRECTION: INCREASE CAL TIME LIMIT: Hrs
UNITS: U/L DECIMAL PRECISON: X.XX
CALCULATION FACTOR:
NO. OF CALIBRATORS: 2 #1: USER DEFINED *

PRIMARY WAVELENTH: 560 nm
SECONDARY WAVELENTH: 700 nm

SAMPLE VOLUME: 4µ L
PRIMARY INJECT RGT:
A: 150 µL
B: 75 µL
SECONDARY INJECT RGT:
None: 0 µL
ADD TIME: 0 SEC

MULTIPOINT SPAN: 1-2: -0.001

REAGENT BLANK
START READ: 288 SEC; END READ: 304 SEC
LOW ABS LIMIT: -1.5; HIGH ABS LIMIT: 1.5
REACTION
START READ; 300 SEC; END READ; 480 SEC
LOW ABS LIMIT: -1.5; HIGH ABS LIMIT: 1.5

USABLE RANGE
LOWER LIMIT: 0.00
UPPER LIMIT: 99999.00

SUBSTRATE DEPLETION
INITIAL RATE: 99.99
DELTA ABS: 1.5

* Entered by operator

Hitachi 917 Parameters
Temperature 37°C

Test	ADA
Assay Code	Rate-A
Assay Point	(39)-(49) **
Wavelength	750/546
Calibration Method	LINEAR
Unit	U/L
Sample Volume	(5) (5)
Reagent vol. R1	(180)(100)(NO)
Reagent vol. R2	(90)(100)(NO)
STD. (1) CONC. – Position	(0)-(1)
STD (1) CONC. – POS	(*)-(2)
ABS Limit	32000-Increase
Expected value (normal value)	4-20
Tech. limit	0-200

* Entered by operator

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