BQ Kits

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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 and plasma samples. For Research Use Only in the USA.

Background
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]

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.

Reagent Preparation & Handling
The ADA reagent comes in a 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 DI water before use. The reconstituted ADA control or calibrator is stable for 1 week at 2-8 °C. Control and additional calibrator is sold separately.

Regent Stability and Storage
Reagents are stable until their expiration date when stored at 2 – 8 °C. Store calibrator separately at -20 °C.

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
Stabilizers	
Reagent 2 (R2) - 25 mL	
Tris HCl, pH 4.0	50mM
Adenosine	10 mM
EHSPT	2 mM
ADA Control	1.0 mL
Adenosine Deaminase (bovine liver) and BSA	

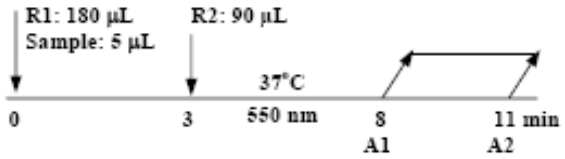
Materials Required but not Provided
- Any instrument with temperature control of 37 +/- 0.5 °C that is capable of reading absorbance accurately at 540nm - 550nm may be used.
- Controls for validations the performance of the Adenosine Deaminase Assay kit.
- Calibrators for the Adenosine Deaminase Assay are provided, should be stored separately at -20 °C.
- 0.9% Saline is needed as Calibrator 0.
- General laboratory equipment.

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



Additional application sheets for use of the Adenosine Deaminase Assay on automated clinical chemistry analyzers are available upon request.

Calibration
A single calibrator, along with 0.9% saline as a zero reference, are needed for calibration. The lot specific calibrator values are stated in the Certificate of Analysis.

Quality Control
BQ Kits recommends that each laboratory use ADA controls (listed under materials required section) to validate the performance of ADA reagents. An ADA control is available from BQ Kits. The control interval and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Each laboratory should follow federal, state, and local guidelines for testing QC material. .

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

Reference Range
Literature cites ADA activity tests in serum samples to be in the range of 0-15 U/L [1-4]. Literature citations show that for pleural fluid[4], values were found to be in the range of 0-30 U/L, and for C.S.F [4], values were found to be in the range of 0-9 U/L.

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.

Analytical Characteristics [5]
These performance characteristics were determined at BQ Kits using automated procedures unless otherwise stated. Results from individual laboratories may vary.

Precision
The precision of the BQ Kits ADA assay evaluated on the Cobas Mira instrument according to a modified 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)	11.11	30.74	9.63	29.62
SD	0.16	0.45	0.47	0.59
Cv %	1.47	1.45	4.90	2.00

Assay Linearity

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

Interference
Assay is not affected by serum bilirubin up to 30mg/dL, hemoglobin up to 200mg/dL, triglycerides up to 750mg/dL, and ascorbic acid up to 4mg/dL.

Safety Precautions and Warnings
1. USA: For Research Use Only. Not for use in diagnostic procedures.
2. EU: For in vitro diagnostic use.
3. Reagent R1 is light-sensitive. It should be stored in a dark place.
4. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those Biosafety in Microbiological and Biomedical Laboratories (HHS publication Number [CDC] 93-8395).
5. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
6. 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.
7. 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)
4. Lakkana B., Sasisopin K: Use of Adenosine Deaminase for the Diagnosis of Tuberculosis: A review J. Infect. Dis Antimicrob Agents 2010; 27:111-8
5. Delacour H., Sauvanet C., Ceppa F., Burnat P.: Analytical performances of the ADA Assay on the Cobas 6000 system. Clinical Biochemistry 43 (2010) 1468-1471.

Hitachi 717 Parameters
Temperature 37°C

Use the following parameters with calibrator and saline for calibration

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

* Each cycle is 12 seconds
* Entered by operator

The above reagent parameter has **not been fully validated** for this analyzer. The parameters are based on BQ Kits' knowledge of the analyzer and the reagents, and should perform adequately. **However, you should use these parameters as guidelines in conjunction with your Quality Control Program for validation.**

Cobas Mira-S Parameters
Temperature 37°C

Use the following parameters with calibrator for calibration.

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		*
STD-2		No

* Entered by operator

Beckman Synchron CX-7 Parameters
Temperature 37°C

Use the following parameters with saline and calibrator for calibration.

CHEMISTRY NAME: Adenosine Deaminase
TEST NAME: [ADA]
CALCULATION FACTOR: 0
REACTON TYPE: RATE 1 MATH MODEL: LINEAR
REACTION DIRECTION: INCREASE CAL TIME LIMIT: Hrs

UNITS: U/L DECIMAL PRECISON: X.XX
NO. OF CALIBRATORS: 2
PRIMARY WAVELENTH: 560 nm
SECONDARY WAVELENTH: 700 nm

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

Calibrators #1: 0; #2 USER DEFINED *

MULTIPOINT SPAN: 1-2: -0.000

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

The above reagent parameter has **not been fully validated** for this analyzer. The parameters are based on BQ Kits' knowledge of the analyzer and the reagents, and should perform adequately. **However, you should use these parameters as guidelines in conjunction with your Quality Control Program for validation.**

Olympus AU 400/600/640/2700/5400 Parameters
Temperature 37°C

Use the following parameters with calibrator and saline for calibration.

Specific Test Parameters

Test Name: * Type: Serum Operation: Yes

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

Reagents: R1 volume 180 µL Dilution 0 µL Min OD Max OD

R2 volume 90 µL Dilution 0 µL L:-2.000 H:2.500

Reagent OD Limit:

Wavelength: Pri. 540 Sec. 700 First L: -2.0000; First H: 2.5000

Method: Rate Last L:-2.0000; Last H: 2.5000

Reaction Slope: + Dynamic Range:

Measuring Point 1: First 20; Last 27 L:-999999.9 H: 999999.9

Measuring Point 2: First Last Correlation Factor:

Linearity 20% A:1.000000 B:0.000000

No-Lag-Time: Onboard stability Period: 28

Calibration Specific

Test Name ++ Type: Serum

Calibration Type 2AB Formula: polygonal Counts: 2 Process: CONC

	Cal No.	OD	CONC	Factor/OD-L	Factor/OD-H
Point 1:	*		0.0	-9999999	9999999
Point 2:	*		*.*	-9999999	9999999
Point 3:					
Point 4:					
Point 5:					
Point 6:					

1-Point Cal. Point: N/A Advanced Calibration: No

MB Type Factor: N/A Calibration Stability Period: 999

* Value input by operator

Hitachi 917 Parameters
Temperature 37°C

Use the following parameters with calibrator for calibration.

** ANALYZE **
TEST NAME : [ADA]
ASSAY/POINT : [Rate A] [10] [27][34][0][0]
WAVE (SUB/MAIN) : [700] [546]
S. VOL. (NORMAL) : [5.0] [0.0] [0]
S. VOL. (DECREASE) : [5.0] [0.0] [0]
S. VOL. (INCREASE) : [5.0] [0.0] [0]
DILUENT : [WATER] [0]
REAGENT VOL (R1) : [180] [0] [xxxxx] [0]
REAGENT VOL (R2) : [0] [0] [xxxxx] [0]
REAGENT VOL (R3) : [90] [0] [xxxxx] [0]
REAGENT VOL (R4) : [0] [0] [xxxxx] [0]
ABS LIMIT : [32000] [Increase] TWIN TESTS: [ ]
PROZONE LIMIT : [-32000] [0][LOWER]
CELL DETERGENT : [Detergent 1]

** CALIBRATION **
CALIB TYPE : [Linear] [ ]
POINT : [2] SPAN POINT [2]
WEIGHT : [0]
AUTO CALIBRATION
TIME OUT CHG. OVER
BLANK : [0] CHANGE LOT: [ ]
SPAN : [0] CHANGE BOTTLE: [ ]
2POINT : [0]
FULL : [0]
SD LIMIT: [100]
DUPLICATE LIMIT: [200]
SENSITIVITY LIMIT: [0]
S1ABS RANGE: [-32000] [32000]

** RANGE **
TEST CODE : [xxx] UNIT: [U/L] DATA MODE [On Board]
CONTROL INTERVAL: [0]
INST. FACTOR (Y=aX + b) : a= [1.0] b= [0.0]
TECHNICAL LIMIT [0] [200]
EXPECTED VALUE [4] [20]
MALE [ ]
FEMALE [ ]

** STANDARD CONCENTRATION **
STANDARD SOLUTION
CONC : [*] [*] [0] [0] [0] [0]
POSITION : [*] [*] [0] [0] [0] [0]
SAMPLE : [5.0] [5.0] [0.0] [0.0] [0.0] [0.0]
PRE-DILUENT
VOLUME : [0.0] [0.0] [0.0] [0.0] [0.0] [0.0]
DILUENT : [0] [0] [0] [0] [0] [0]
CALIB CODE : [*] [*] [0] [0] [0] [0]

* Value input by operator