

## Adenosine Deaminase Assay Kit

Catalog Number: BQ014EALD

Method: Colorimetric assay (Kinetic)

Wavelength: 550nm

Linear range: 0-200 U/L

Stable two liquid reagents system

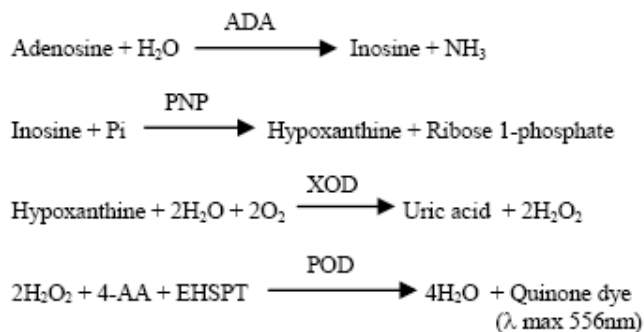
### Intended Use

Adenosine deaminase (ADA) assay kit is for determination of ADA activity in human serum samples.

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  $\gamma$ -GT (GGT) tests. ADA assay may also be useful in the diagnostics of tuberculous pleuritis (3). In U.S., this product is for research use only.

### 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.



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

### Reagent Table (275 tests)

Reagent 1 (R1) 50 mL	50 mM Tris-HCl pH 8.0 2 mM 4-AA 0.1 U/mL PNP 0.2 U/mL XO 0.6 U/mL Peroxidase Stablizers
Reagent 2 (R2) 25 mL	50mM Tris-HCl pH 4.0 10 mM Adenosine 2 mM EHSPT
ADA Control 1.0 mL	Adenosine deaminase (bovine liver) and BSA

Reagents are ready-to-use and stable for 1 year when stored at 2 – 8 °C. ADA Control is in lyophilized form, need to be reconstituted with 1.0 mL of water before use. The reconstituted ADA control is stable for 1 week at 4 °C. Control is sold separately.

### Sample Specimen

Use fresh and non-hemolyzed serum or plasma for ADA test. ADA is stable for one week at 4 °C.

### Assay Procedure

1. Parameter settings:

Method: Kinetics                      Temperature: 37 °C  
Wavelength: 550 nm                  Reaction time: 10 min  
Sample/Reagent: 1: 54

Use water to blank (autozero) cuvette at 550 nm

2. Assay:

- Reagents R1 and R2 are pre-equilibrated to room temperature prior to the assay. R1 is light sensitivity.
- Mix 180  $\mu$ L of R1 and 5  $\mu$ L of plasma sample.
- Incubate at 37°C for 3 or 1.5 min.
- Add 90  $\mu$ L of R2, and incubate for 5 min followed by monitoring the absorbance at 550 nm for 3 min with 1 min interval to obtain  $\Delta A/\text{min}$  values.

- Calculate the average rate of the absorbance change  $\Delta A/\text{min}$ .

$$\Delta A/\text{min} = \frac{(\Delta A_1/\text{min} + \Delta A_2/\text{min} + \Delta A_3/\text{min})}{3}$$

- Calculate ADA activity (U/L) in the plasma sample by using the formula:

$$\text{ADA (U/L)} = \frac{\Delta A/\text{min} \times Tv}{\epsilon \times Sv \times L} = \Delta A/\text{min} \times 1708$$

Note:

Before performing the assay in lab instrument or analyzer, users should verify the accuracy of the calculation factor. The calculation Factor for UV spectrophotometer is 1708 when the cuvette path length is 1 cm. Users should determine the calculation factor for the specific instrument being used in the lab based on cuvette pathlength and other conditions. This can be done experimentally as follows:

- 1) Bio-Quant controls with known values are run in triplicate
- 2) The calculation factor is modified so that the result matches Bio-Quant control target values

Bio-Quant ADA controls can be purchased separately.

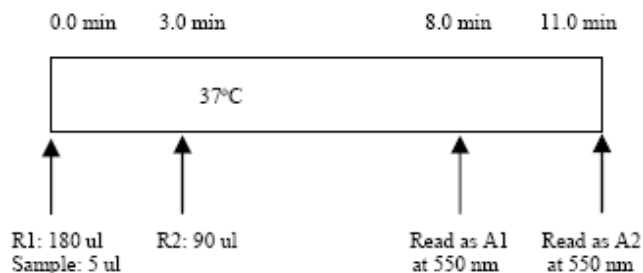
$\epsilon$ :  $\mu$ molar extinction coefficient of quinone dye ( $\epsilon = 32.2 \times 10^{-3} \mu\text{M}^{-1}\text{cm}^{-1}$ )

Tv: Total reaction volume (mL)

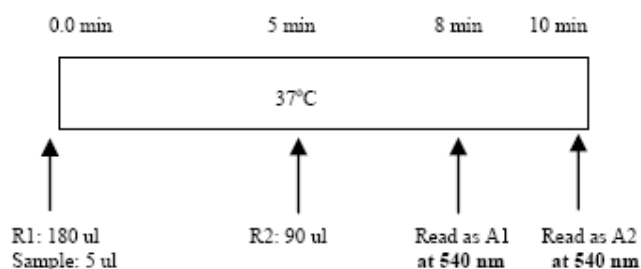
Sv: Sample volume (mL)

L: Cuvette light path length (1.0cm)

Assay procedure is depicted as the scheme shown below:



For instruments that allow a total of 10min reaction time at 540nm, the assay procedure can be modified as below:



### Assay Linearity

The assay is linear from 0-200 U/L ( $r^2 > 0.99$ ).

Precision: Intra assay CV% < 4.5%, Inter assay CV% < 6.0%

If the sample 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.

### Stability

The reagent (R1) is stable for 1 year if stored at 2 – 8 °C in dark.

### Reference Value

Healthy subjects have a ADA activity in the range of 4-20 U/L, or 66 – 332 nkat/L. It is recommended that each laboratory should establish its own range of reference values.

### Product Features

- Liquid two reagents system, ready-to-use for both manual method and automated chemistry analyzers (Kinetics)
- Assay is specific for ADA and has no detectable reaction with other nucleosides
- Assay is not affected by serum bilirubin up to 20mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 750mg/dL, and ascorbic acid up to 4 mg/dL.

### Safety Precautions and Warnings

For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Reagent 1 (R1) contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

### References

1. Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. *Am. J. Gastroenterol.* 88: 266-271 (1993)
2. Kalkan 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 diagnostic tool for tuberculous pleurisy. *Thorax* 50: 672-674 (1995)

Cobas Mira-S Parameters	ADA DZ117A
Measurement Mode	Absorb
Reaction Mode	R-S-SR1
Calibration Mode	Factor
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 <sub>2</sub> O
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
Factor	<b>3416</b>

\* Each reading cycle is 25 seconds

HITACHI 717 Parameters	ADA DZ117A
Test	ADA
Assay Code	Rate-A
Assay Point	(39)-(49)**
Wavelength	750/546
Calibration Method	K Factor
STD. (1) CONC.-Position	(0)-(1)* (blank)
Unit	U/L
Sample volume	(5)(5)
Reagent vol. R1	(180)(100)(NO)
Reagent vol. R2	(90)(100)(NO)
<b>K Factor</b>	<b>2420</b>
ABS Limit	32000-Increase
Expected value (normal Value)	4-20
Tech. Limit	0-200

Attention: \* Entered By Operator

\*\* Each reading cycle is 12 seconds.

Instrument Parameters for Olympus AU 400  
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 MB 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: **2600.000** Calibration Stability Period: 999

**Bechman Synchron CX-7 Delta Parameters**  
Temperature 37°C

CHEMISTRY NAME: Adenosine deaminase

TEST NAM: [ ADA ]

REACTION TYPE: RATE 2 MATH MODEL: LINEAR

REACTION DIRECTION: INCREASE CAL TIME LIMIT: Hrs

UNITS: U/L DECIMAL PRECISION: X.XX

CALCULATION FACTOR: **3350**

NO. OF CALIBRATORS: 0

PRIMARY WAVELENGTH: 560 nm

SECONDARY WAVELENGTH: 700 nm

SAMPLE VOLUME: 5 µL

PRIMARR INJECT RGT:

A: 180 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