

## Adenosine Deaminase Assay Kit (ADA)

**Method:** Enzymatic Method

Cat. No.	Size	Instrument
GB8000G	R1: 2 x 20 ml R2: 1 x 20 ml	For Hitachi 717 & ShimadzuCL7200/8000
GS8001G	R1: 2 x 20 ml R2: 1 x 20 ml	For Hitachi917 & OlympusAU640/400/600
GX8001G	R1: 1 x 40 ml R2: 1 x 20 ml	For SYNCHRON CX4-5- 7-9/LX20/DXC600-800
GB8000G/B	R1: 3 x 40 ml R2: 3 x 20 ml	For Hitachi 717 & ShimadzuCL7200/8000
GS8001G/B	R1: 3 x 40 ml R2: 3 x 20 ml	For Hitachi917 & OlympusAU640/400/600
GD8001G	R1: 8 x 3.7 ml R2: 4 x 3.7 ml	For DATE DEMENSION
GT8001G	R1: 2 x 20 ml R2: 1 x 20 ml	For TOSHIBA

### INTENDED USE

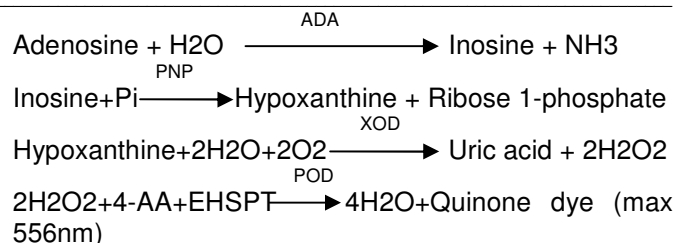
Adenosine deaminase (ADA) assay kit is for determination of ADA activity in human serum, plasma, pleural fluid and cerebrospinal fluid (C.S.F.) samples. For investigational use only.

### CLINICAL SIGNIFICANCE<sup>[1,2,3]</sup>

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. Increased ADA activity was also observed in patients with tuberculous effusions. 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.

### 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<sub>2</sub>O<sub>2</sub>) by xanthine oxidase (XOD). H<sub>2</sub>O<sub>2</sub> is further reacted with TOOS 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.

### SAMPLE COLLECTION AND PREPARATION

Use fresh and non-hemolyzed serum or plasma, Plural fluids and CSF for investigation. If fluids are viscous in nature, then kindly centrifuge it well and use supernatant of the same. ADA is stable for one week at 4°C.

### REAGENT COMPOSITION

Contents	Concentration
R1	
Tris-HCl pH 8.0	25 mmol/L
4-AA	4 mmol/L
PNP	0.3 U/mL
XO	0.4 U/mL
Peroxidase	0.1 U/MI
Stabilizer	
R2	
Tris-HCl pH 4.0	25 mmol/L
Adenosine	11 mmol/L
EHSPT	4 mmol/L

### STABILITY AND PREPARATION OF SOLUTIONS

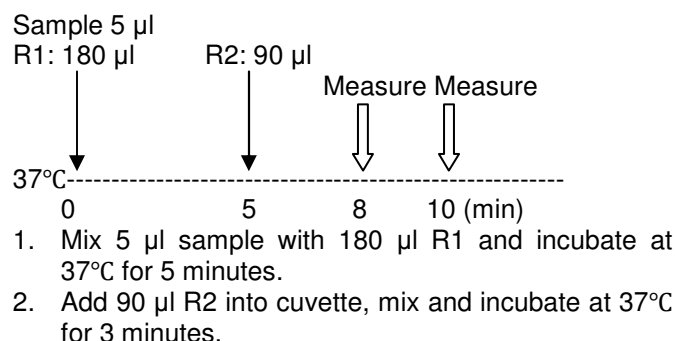
Reagents are ready to use.

Stable up to the expiry date when stored at 2-8°C. Once opened the reagent is stable for 1 month onboard the analyser at approximately 2-8°C.

### ASSAY PROCEDURE

Assay Mode: Kinetics

Wave Length (main/sub): 546 nm/800 nm



# Gcell

3. Read initial absorbance and start timer simultaneously, read again after 1 and 2 minutes.
4. Calculate absorbance change per minute ( $\Delta A/\text{min}$ )

## CALCULATION

$$\text{Concentration} = \frac{\Delta A_{\text{sample}} / \text{min}}{\Delta A_{\text{calibrator}} / \text{min}} \times \text{Calibrator value}$$

## CALIBRATION

Recommend that this assay should be calibrated using Gcell Calibrator (Cat. No. GCADA).

## QUALITY CONTROL

For quality control, use ADA control as daily quality control sera and can be purchased separately. Values should fall within a specific range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.
4. Check the quality of the water used for reagents reconstitution.

## NORMAL VALUE

The ADA activities in 60 healthy human serum samples were found to be in the range of 4-20 U/L. For pleural fluid, values were found to be in the range of 0-40 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 reference range to reflect the age, sex, diet and geographical location of the population

## SPECIFIC PERFORMANCE CHARACTERISTICS

### INTERFERENCE

The following analytes were tested up to the levels indicated and found not to interfere:

Hemoglobin	up to 800 mg/dl
Intralipid	up to 1000 mg/dl
Ascorbic acid	up to 50 mg/dl

## PRECISION

The CV of the test should be less than 5%.

## LINEARITY

The method is linear up to 176 U/L. Sample above this concentration should be diluted with 0.9% NaCl and reassay. Multiply the result by dilution factor.

## SENSITIVITY

The minimum detectable concentration of ADA with an acceptable level of precision was determined as 4 U/L.

## CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

$Y = 0.9766X + 0.0206$ , and a correlation coefficient of 0.9989, 50 patient samples were analyzed.

## SAFETY PRECAUTIONS AND WARNINGS

1. For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
2. Solution 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.
3. 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.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

## 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).

Intra assay precision		
N=20	Level1	Level 2
Mean	29.0	140.4
SD	0.17	0.29
CV	0.59%	0.20%
Inter assay precision		
N=5	Level1	Level 2
Mean	28.6	135.7
SD	0.12	1.10
CV	0.42%	0.81%

