Bioway Chemistry Reagent Series

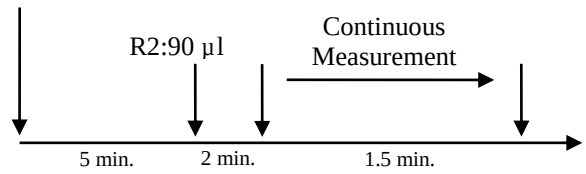
The Adenosine Deaminase Reagent Kit

Detection of Adenosine Deaminase in Human Serum or Plasma on Chemistry Analyzers

Cat. No. R002K11 ***The ADA Reagent Kit***

SUMMARY OF TEST PROCEDURE

Figure 1: Procedure Diagram



*Refer to Figure 1 and the package insert for detail

Table 1: Instrument Parameters*

Calibration method	K factor	Slope of reaction	Increase
Testing wavelength	Dλ : 550 nm Sλ : 700 nm	Sample volume	5 µl
Test method	Rate Method	R1 volume	180 µl
Reaction temperature	37℃	R2 volume	90 µl

INTENDED USE

Bioway Chemistry Reagent Series **ADA Reagent Kit** (the Kit) is an assay intended for *in vitro* quantitative detection of Adenosine Deaminase in human serum or plasma on automated clinical chemistry analyzers.

SUMMARY AND EXPLANATION

Adenosine Deaminase is an enzyme involved in purine metabolism, catalyzing the deamination reaction from adenosine to inosine. It can be found in all mammalian cells, especially in immune cells. There are two different isoforms of ADA: ADA1 and ADA2. The two isoforms regulate the ratio of adenosine to deoxyadenosine and influence the potential of killing parasites. ADA2 is the major form existing in human plasma and shows increased in many diseases. ADA activity is elevated in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, viral hepatitis, hepatoma or tuberculous effusions. ADA can also be used in the workup of lymphocytic pleural effusions or peritoneal ascites, in those specimens with low ADA levels essentially excludes tuberculosis from consideration.

TEST PRINCIPLES

The Kit utilizes enzymatic and kinetic reactions to measure the ADA activity (U/L) in human serum or plasma.

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

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

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

$$H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 2H_2O + \text{Quinone dye}$$

Adenosine is converted to inosine then hypoxanthine by the series deamination with adenosime deaminase (ADA) and purine nucleoside phosphorylase (PNP). Hypoxanthine then reacts with water and oxygen and forms uric acid and hydrogen peroxide. In the end hydrogen peroxide is reduced to water and quinone dye is produced by reacting with 4-aminoantipyrine and N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT). The process is quantified by measuring the absorabances at 550 nm in a kinetic fashion.

The rate of increase in absorbance at 550 nm is directly proportional to the ADA activity in the sample.

MATERIALS PROVIDED

Reagents:

R1	Glycine buffer pH=7.2 Xanthine oxidase Nucleoside phosphorylase	80 mmol/L 800 U/L 50 U/L
R2	4- aminoantipyrine Adenosine Peroxidase EHSPT	2.0 mmol/L 10 mmol/L 600 U/L 2 mmol/L

MATERIALS NEEDED BUT NOT PROVIDED

1. Automated chemistry analyzer
2. ADA control and calibrator set (available for purchase)

INSTRUMENT

The Kit is applicable on most automated chemistry analyzers. Refer to specific instrument application for suggested settings.

STORAGE AND STABILITY

Store the reagents at 2-8℃. Avoid direct sunlight. The Kit is stable through the expiration date when stored properly. The reagent is stable for 1 month at 2-8℃ after opening.

PRECAUTIONS

1. The Kit is for *in vitro* diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.

SPECIMEN COLLECTION AND HANDLING

Follow standard laboratory procedures to collect serum preventing hemolysis.
It is recommended to perform test immediately after sample collection.

TEST PROCEDURE (see Figure 1)

Reagent 1 and 2 are liquid stable ready-to-use, no preparation needed.

Calibration: Recommend using Bioway calibrator set for optimal results.

Test procedure: see Figure 1 and Table 1 for instrument parameter setup. Refer to specific instrument application for suggested setting.

1. Add 5 µl of sample and 180µl of R1; mix well and incubate at 37℃ for 5 minutes.
2. Add 90µl of R2; mix well and incubate at 37℃ for 2 minutes.

3. Take continuous optical density measurement for 1.5 minutes.
4. Calculate average Δ A /min

RESULT

The ADA activity in U/L can be obtained by the following calculation:

$$\frac{\text{Abs. sample}}{\text{Abs. standard}} \times \text{Standard Conc. (U/L)} = \text{ADA Conc. (U/L)}$$

Please refer to instrument application if testing under different conditions.

EXPECTED VALUES

≦30U/L

It is recommended for each laboratory to establish its own expected values

QUALITY CONTROL

Using Bioway controls with known concentration is recommended before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified.

LIMITATIONS

1. The Kit is for *in vitro* use on automated chemistry analyzers only.
2. The test result from the Kit should not be used as the only basis for definite diagnosis.
3. Samples with ADA exceeding the maximum measurement range should be diluted with saline and retested.

PERFORMANCE CHARACTERISTICS

Linearity: 0 - 200 U/L (R≥0.990)

Accuracy: Bias proportion 90%~110%

Precision: Within Run: CV≤6%;
Run-to-Run: CV≤8%

Interference: no interference detected for: ascorbic acid (≤40mg/dl), chylomicrons (≤3000NTU), conjugated bilirubin (≤40mg/dl), and unconjugated bilirubin (≤40mg/dl).

Reagent Blank Absorbance: at 550nm wavelength and 10 mm optical diameter, O.D. ≤ 0.1

REFERENCES

1. Lamsal M *et al.*, Southeast Asian J Trop Med Public Health. 38(2):363-9 (2007)
2. Riquelme A *et al.*, J Clin Gastroenterol. 40(8):705-10 (2006)
3. D. Jiménez Castro et al., European Respiratory Journal, 21(2):220-224 (2003)

Not Intended for Sale in the United States.

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