WELDONBIOTECH

Inspiration for life science

Wel- Chem *Adenosine Deaminase Kit*

Configuration

The Wel-Chem Adenosine Deaminase reagent is provided in following kit configuration:

Instrument	Catalog No.	Kit Size(75ML)
Universal	ADA-237WB	R1: 1 x 50 mL R2: 1 x 25 mL
Calibrator	Liquid stable	Cal:1x1mL
Control	Liquid stable	Con:1x2 mL

Instrument	Catalog No.	Kit Size(50 ML)
Universal	ADA-137WB	R1: 1 x 33.3 mL R2: 1 x 16.7 mL
Calibrator	Liquid stable	Cal:1x1mL
Control	Liquid stable	Con:1x2 mL

Calibrator and control sold separately

Intended Use

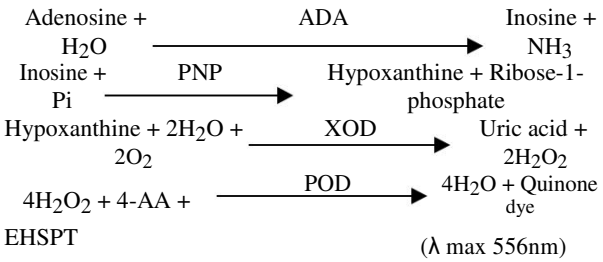
Adenosine deaminase (ADA) assay kit is for determination of ADA activity in human serum, plasma,

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]

Assay Principle

The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nu-cleoside 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 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 Composition

Active Ingredients	Concentration
Reagent 1	
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	
Tris-HCl, pH 4.0	50 mM
Adenosine	10 mM
EHSPT	2 mM
ADA Control	
Adenosine deaminase (bovine liver) and BSA	

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 and calibrator sold separately.

Reagent Stability and Storage

Reagents are stable until their expiration date when stored at 2-8°C.

Specimen Collection and Handling

Serum or heparinized plasma may be assayed. Ideally, venous blood should be collected and handled an aerobically. Do not use citrate or oxalate as anticoagulant.

Plasma and serum, after prompt separation from cells or clot, should be kept tightly stopped. ADA content of blood is stable for 1 week when stored at 2–4°C.

Precautions

1. Reagent R1 is light-sensitive. Store in a dark place.
2. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Bio- medical 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

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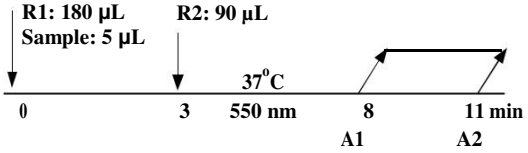
4. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
5. The reagents contain < 0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
6. Do not use the reagents after the expiration date labeled on the outer box.
7. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

Assay Procedure

Test Scheme for Chemistry Analyzers



Application sheets for use of Weldon 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, should be used as directed to calibrate the procedure.

Quality Control

Weldon recommends that each laboratory use ADA controls to validate the performance of ADA reagents. An ADA control is available from Weldon. 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 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.

Performance Characteristics

These performance characteristics were determined at Weldon using automated procedures unless otherwise stated.

Precision

The precision of the Weldon Adenosine Deaminase Assay was evaluated on the Cobas Mira instrument according to Clinical Laboratory Standards Institute (formerly NCCLS) 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
C_V%	1.4	1.8	2.13	2.13

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-bin up to 200 mg/dL, triglycerides up to 750mg/dL, and ascorbic acid up to 4 mg/dL.

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)

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