ADENOSINE DEAMINASE

(ENZYMATIC METHOD)

ENZOPAK

Last update 09-2020

Ref. CC3-ADA.025, 1x20 ml
CC3-ADA.25U, 2x20 ml

INTENDED USE

Adenosine Deaminase Reagent is an enzymatic assay intended for in vitro quantitative detection of Adenosine Deaminase in human serum on semi and fully automated clinical chemistry analyzers.

DIAGNOSTIC 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. 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 γ-GT (GGT) tests. ADA assay may also be useful in the diagnostics of tuberculous pleuritis.

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 TOOS and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (POD) to generate quinoneimine dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.

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

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

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

$$2H_2O_2 + \text{4-AAP} + \text{EHSPT} \xrightarrow{\text{POD}} 4H_2O + \text{Quinoneimine dye}$$

One unit of ADA is defined as the amount of ADA that generates one µmol of inosine from adenosine per min at 37℃.

PRESENTATION

	No of bottle / Vial	
Store all reagents at 2 to 8°C	1x20 ml	2x20 ml
• 1 - Adenosine Deaminase	1x15 ml	2x15 ml
• 2 - Adenosine Deaminase	1x5 ml	2x5 ml
• Adenosine Deaminase Calibrator (value stated on vial label)	1	1

FINAL REAGENT COMPOSITION

	Concentration
• Tris-HCl	50mmol
• 4-AAP	2mmol
• PNP	0.1U/mL
• XOD	0.2U/mL
• Tris-HCl	50mmol
• Peroxidase	0.6U/mL
• Adenosine	10mmol
• EHSPT	2mmol
• Sucrose	1%
• BSA	1g/L

PRECAUTION

1. The results are only for clinical reference. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment reactions.
2. The reagent is only for in vitro diagnosis use, not oral administration.
3. To detect the same sample, the result shall be different from different manufacturers.
4. All samples and reaction wastes should be treated as infectious sources and operators should take necessary protective measures.
5. The reagent contains preservatives to avoid touching the skin and mucosal tissue. If you are accidentally spilled, please rinse it with clean water immediately and go to the hospital if necessary.
6. The reagent and sample volume shall be adjusted in proportion to the requirements of different biochemistry analyzers.

PREPARATION OF WORKING REAGENT

The reagents are liquid and ready to use.

REAGENT STORAGE AND STABILITY

The reagents shall be stored at 2-8℃. Do not freeze. The reagents and calibrator are stable when stored as instructed until the expiration date on the label. The on-board stability shall be 30 days.

ADA calibrator shall be stored at 2-8℃. The calibrator is lyophilized powder. The liquid after reconstitution is stable up to 3 days when stored at 2-8℃.

SPECIMEN COLLECTION

Non hemolytic serum.

CALIBRATION

ADA Calibrator should be dissolved with 1mL purified water before using, and wait for 5-10 minutes to completely dissolve. After gently mixing, it can be used as the original calibration solution.

If you use other manufacturer's calibrator, please verify by yourself. Please do re-calibration if the reagent lot number change, quality control drift, instrument maintenance or important parts replacement.

QUALITY CONTROL

Quality control should be carried out before samples are tested every day to ensure the stability of the test system. Using commercially available 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.

The results of quality control should be within the allowable range. If the results deviate from the scope, please take the following steps to find out the reasons,

1. Check whether the parameter setting and the light source are correct.
2. Check the cleanliness of the analyzer cup and sampling needle.
3. Check whether the water is contaminated or not. Bacterial growth can lead to incorrect results.
4. Check the reaction temperature and validity of the reagent.

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REACTION PARAMETERS

- Type of Reaction : kinetic (Increase)
- Wavelength-1 : 546nm
- Wavelength-2 **(Optional)** : 700nm **(Optional)**
- Flow cell temperature : 37^0C
- Incubation : 300 Sec.
- Delay Time : 180 Sec.
- Interval Time : 60 Sec.
- No. of Reading : 2
- Calibrator Concentration : As mentioned on vial
- Light Path : 1 cm
- Zero setting with : Distilled water

TEST PROCEDURE FOR FULLY AUTO ANALYZER

Sample Volume	6 uL
Reagent 1 (R1)	225 uL
Mix Sample and R1 well and incubate for 5 minutes at 37°C, then add:	
Reagent 2 (R2)	75 uL
Mix well and incubate for 3 minutes at 37°C, read absorbance value continuously for 2 minutes then calculate Δ A/min.	

FOR SEMI AUTO ANALYZER

Sample Volume	10 uL
Reagent 1 (R1)	375 uL
Mix Sample and R1 well and incubate for 5 minutes at 37°C, then add:	
Reagent 2 (R2)	125 uL
Mix well and incubate for 3 minutes at 37°C, read absorbance value continuously for 2 minutes then calculate Δ A/min.	

TEST RESULTS

ADA (U/L) = ΔA/min × F*.

$$\text{Factor} = \frac{Tv \times 1000}{\varepsilon \times Sv \times L}$$

ε: mmolar extinction coefficient
Tv: Total reaction volume (mL)
Sv: Sample volume (mL)
L: Cuvette light path length (1.0cm)

LIMITATIONS FOR INTERFERENCE

If the concentration of interfering substances in the sample satisfies the following requirements, the test results will not be affected

Bilirubin (≤60mg/dL)
Hemoglobin (≤50mg/dL)
Intralipid (≤750mg/dL)

NORMAL VALUES

4-24 U/L

It is recommended that each laboratory should establish its own reference range.

EXPLANATIONS OF TESTING RESULT

Professionals are responsible for the audit of inspection results. The test results will be affected by age, sex, weight and so on. Usually, the results are considered normal within the reference range. If the results out of the range, they should be re-determined for confirmation. And if they are clearly beyond the reference range or even beyond the reference range after confirmation, the target content in serum is considered abnormal. If the test results are inconsistent with or even contrary to the clinical situation, we should analyze and find out the reasons.

The increase of ADA activity in serum can be seen in acute hepatitis, alcoholic liver fibrosis, chronic active hepatitis, cirrhosis, viral hepatitis, and also in tuberculosis.

PERFORMANCE CHARACTERISTICS

1. **Appearance:**
   R1: Colorless to yellowish clarifying liquid.
   R2: Colorless to yellowish clarifying liquid.
   ADA Calibrator: White to yellowish lyophilized powder. Colorless to yellowish liquid after dissolving.
2. **Linearity:**
   In the linear range of 2-200 U/L, the linear correlation coefficient $R^2 \geq 0.990$
3. **Precision:** CV≤5%
4. **Inter-batch deviation:** R≤10%
5. **Accuracy:** The measured value shall be fallen within the range of quality control target value.
6. **Analytical sensitivity:** At 546nm and optical diameter of 1cm, when the activity of ADA in the sample is 15u/ L, the absorbance change rate of reagent and sample is $0.005 \leq (\triangle A / min) \leq 0.050$.
7. **Blank absorbance:** At 546nm and 1cm optical path, reagent blank absorbance A≤0.200.

REFERENCES

1. Lamsal M etal., 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.Jimenez Castro et al., European Respiratory Journal, 21(2):220-224(2003)

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