# LiquiMAX ADA-Kinetic

**Enzymatic - Kinetic Method**

**Intended Use:** Adenosine deaminase (ADA) assay kit is for determination of ADA activity in human serum, plasma, pleural fluid, pericardial fluid, ascitic fluid

**Order Information**

| Ref./Cat. No. | Pack Size | Presentation |
|---|---|---|
| AVADA (K) - 10 | 10 ml | (R1 : 10 x 0.75 ml, R2 : 2 x 2 ml) |
| AVADA (K) - 25 | 25 ml | (R1 : 2 x 9 ml, R2 : 2 x 4 ml) |

**PRODUCT FEATURES:**

1. Two Liquid Reagents
2. Kinetic Method. (5 Minutes First Incubation+ 5 Minutes Second Incubation)
3. Linearity : 200 IU/L
4. Kinetic reaction time 300 sec (60 Sec + 240 Sec.).
5. Tailor made for tropical Conditions.
6. Incorporates Six Sigma methodologies throughout the manufacturing processes wherein the product under goes various stringent process checks like Defining, Measuring,Analyzing, Improving and Controlling. (DMAIC)
7. Assay is not affected by Serum Bilirubin up to 20 mg/dL, Hemoglobin up to 200 mg/dL, Triglycerides up to 750 mg/dL, and Ascorbic acid up to 4 mg/dL.
8. Can be used on any discrete semi automated and automated analyzers.

**SUMMARY AND CLINICAL IMPORTANCE:**

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 y-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 (H202) by xanthine oxidase (XOD). H202 is further reacted with N-Ethyl-N-(2-hydroxy-3- sulfopropyl)-3methylaniline (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.

$Adenosine + H_2O \xrightarrow{ADA} Inosine + NH_2$

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

$Hypoxanthine + 2H_2O + 20_2 \xrightarrow{XOD} Uric\,Acid + 2H_20_2$

$H_20_2 + 4\text{-}AA + EHSPT \xrightarrow{POD} 2H_20 + Quinone\,Dye\ (\ max\ 546nm)$

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

**STABILITY AND STORAGE**

When stored at 2-8°C the reagents are stable until the expiration date stated on the bottle and kit box labels. It is recommended that when the reagent is not in use for prolonged periods of time the reagent be capped and stored at 2-8°C.

**SPECIMEN COLLECTION AND HANDLING:**

**Collect specimen prior to use of antimicrobial agent. Wherever possible indicate clearly that the patient is on anti tubercular drugs**

**Serum / Plasma :** No special preparation of the patient is required prior to sample collection by approved techniques It is recommended to use fresh sample specimen for testing. Do not use hemolyzed, contaminated or turbid sample specimens. Serum or EDTA, heparinized plasma may be used. Ideally, venous blood should be collected and handled anaerobically. **Do not use citrate or oxalate as anticoagulant.**

Plasma and serum, after prompt separation from cells or clot, should be kept tightly stoppered.

**Body Fluids:** Disinfect the site and collect specimen with aseptic precaustions

**Storage:** ADA samples are stable for 3 days at 2-4°C or 7 Days at -20°C.

**ASSAY PROCEDURE: (Serum,Plasma, Pleural Fluid, Pericardial and Ascitic Fluid)**

| | | |
|---|---|---|
| **R1** | **700 µl** | |
| **Sample Serum,Plasma, Pleural Fluid, Pericardial and Ascitic Fluid)** | **50 µl** | **First Incubation (In the incubator)** |
| **Mix well and Incubate for 5 minutes at 37°C in an incubator** | | |
| **Add R2** | **300 µ** | **Second Incubation (In the Analyzer)** |

Immediately aspirate in to the analyzer whose settings are as follows.

measure the change of optical density during the next 300 seconds ( 5 Minutes) against distilled water at 546 nm as follows depending on the analyzer settings

Delay: 60 Sec
Measuring: 240 Sec — Total time 300 sec (In some Analyzers)

(OR)

Delay: 60 Sec
Kinetic Interval: 60 Sec — Total time 300 sec (In some Analyzers)
Number of readings: 5

**Calculation**

From the absorbance reading calculate AA/min and multiply by the corresponding factor

**ADA activity (IU/ml) = D A/Min x Factor** (Printed on & inside the kit) **(Serum,Plasma, Pleural Fluid, Pericardial and Ascitic Fluid)**

**System Parameters: (Serum,Plasma, Pleural Fluid, Pericardial and Ascitic Fluid)**

| | | |
|---|---|---|
| Reaction Type (Mode) | Kinetic | |
| Reaction Direction | Increasing | |
| Wave Length | 546nm | |
| Flow Cell Temp. | 37°c | |
| Zero Setting with | Distilled Water | |
| Delay time | 60 seconds | (OR) Delay time : 60 Sec |
| Kinetic Interval | 60 seconds | Measuring time : 240 Sec |
| Number of readings | 5 | |
| Reagent Volume (R1+R2) | 700 ul+ 300 µl | |
| Sample Volume | 50 µl | |
| Factor | Printed on & inside the kit | |
| Linearity | 200 | |
| Units | U/L | |

**ASSAY PROCEDURE: CSF**

| | | |
|---|---|---|
| **R1** | **700 µl** | |
| **CSF Sample** | **200 µl** | **First Incubation (In the incubator)** |
| **Mix well and Incubate for 5 minutes at 37°C in an incubator** | | |
| **Add R2** | **300 µl** | **Second Incubation (In the Analyzer)** |

Immediately aspirate in to the analyzer whose settings are as follows.

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measure the change of optical density during the next 300 seconds ( 5 Minutes) against distilled water at 546 nm as follows depending on the analyzer settings

| | | |
|---|---|---|
| Delay: | 60 Sec | |
| Measuring: | 240 Sec | Total time 300 sec (In some Analyzers) |
| (OR) | | |
| Delay: 60 Sec | | |
| Kinetic Interval: 60 Sec | | Total time 300 sec (In some Analyzers) |
| Number of readings: 5 | | |

**Calculation**

From the absorbance reading calculate AA/min and multiply by the corresponding factor

**ADA activity (IU/ml) = ΔA/ Min x Factor (Printed on & inside the kit)**

**System Parameters:**

| | | |
|---|---|---|
| Reaction Type (Mode) | Kinetic | |
| Reaction Direction | Increasing | |
| Wave Length | 546nm | |
| Flow Cell Temp. | 37ºC | |
| Zero Setting with | Distilled Water | |
| Delay time | 60 Seconds | (OR) : 60 Sec |
| Kinetic Interval | 60 Seconds | Measuring : 240 Sec |
| Number of readings | 5 | |
| Reagent Volume (R1+R2) | 700 ul+300µl | |
| Sample Volume | 200 | |
| Factor | Printed on & inside the kit | |
| Linearity | 200 | |
| Units | U/L | |

**QUALITY CONTROL**

Control sera are recommended to monitor the performance of assay procedures

**REFERENCE VALUES: '**

| | | |
|---|---|---|
| **Serum, Plasma, Pleural, Pericardial and Ascitic Fluids:** | **Normal:** | **< 40 U/L** |
| | **Suspect:** | **> 40 - 50 U/L** |
| | **Positive:** | **> 50 U/L** |
| **CSF :** | **Normal:** | **< 10 U/L** |
| | **Suspect:** | **10-11 U/L** |
| | **Positive:** | **>11 U/L** |

**(It is recommended that each laboratory should establish its own reference range representing its patient population)**

**PERFORMANCE DATA**

The following data was obtained using the LiquiMAX ADA -Kinetic on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

**Sensitivity:** The minimum detectable is 10 U/L

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**IMPRECISION**

| **Within-run Precision:** | **Mean (U/L)** | **+2s** | **CV%** |
|---|---|---|---|
| **Serum** | **18.2** | **+0.28** | **2.54** |
| **Serum 2** | **119.8** | **+6.9** | **0.84** |

**Run-to-run (Day-to-day) Precision:**

| | **Mean (U/L)** | **+2S** | **CV%** |
|---|---|---|---|
| **Serum 1** | **8.3** | **+0.38** | **2.92** |
| **Serum 2** | **120.6** | **+7.6** | **1.02** |

Correlation : A group of 20 Samples from 15 to 130 U/L was assayed by this procedure and using a similar commercially available ADA Reagent. Comparison of the data gave following results: Linear regression equation y = 1,0332x + 0,43 Correlation coefficient r = 0,9968

**LINEARITY**

When run as recommended the assay is linear up to ADA 200 IU/L. Specimens with ADA concentrations greater than 200 IU/L should be diluted with ammonia free water and reassayed. Multiply results by the dilution factor.Linearity on various automated instruments may vary from this value. The user should consult the specific instrument application.

**Notes:**

1) **Patients with Hyperamoniemia, Kidney disorders and Hepatitis can present high levels of ADA.Patients with chronic malnutrition or HIV can present low levels of ADA.**
2) **High levels of ADA are also found in Leprosy, Brucellosis, HIV Infections, Viral Hepatitis, Infectious Mononucleosis and Liver Cirrhosis. Before arriving at a diagnostic decision, these clinical conditions must be ruled out.**
3) **Using a Cut Off Level of 60 IU/L ADA values have been reported to show the specificity and sensitivity of the test as above 90% for the MTB Infection.**
4) **Below 60 IU/L of ADA, the Plasma ADA specificity and sensitivity is lower and should be interpreted in the light of other tests for confirmation of Mycobacterium tuberculosis infection.**

**REFERENCES**


1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).
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**for advanced diagnostic solutions you can count on AVECON**