DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
A – 2351 Wiener Neudorf, Austria, IZ-NÖ Süd, Hondastrasse, Objekt M55
Phone: ++43 (0) 2236 660910-0, Fax: ++43 (0) 2236 660910-30, e-mail: office@dialab.at

Liquid Reagents – ready to use

ADENOSINE DEAMINASE (ADA)

Enzymatic, colorimetric
2 Reagents

Diagnostic reagent for quantitative in vitro determination of adenosine deaminase (ADA) in human serum, plasma, pleural fluid, and cerebrospinal fluid on photometric systems

REF	Kit Size	Content
913810B	**1 x 1 L**	1 x 0.667 L R1 + 0.333 L R2
913816	**6 x 25 mL**	4 x 25 mL R1 + 2 x 25 mL R2
913813	**3 x 25 mL**	2 x 25 mL R 1 + 2 x 12.5 mL R2

Additionally offered:

913870SV	1 x 1 mL	ADA Calibrator
913880	2 x 1 mL	ADA Control Set (2 levels)

TEST PARAMETERS

Method:	Colorimetric, 2-point kinetic, increasing reaction, enzymatic
Wavelength:	550 nm
Temperature:	37 °C
Sample:	Serum, heparinized plasma, pleural fluid, cerebrospinal fluid
Linearity:	up to 200 U/L
Sensitivity:	Limit of detection: 0.03 U/L

SUMMARY

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. Published literature states that 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].
These reports state that 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 and may also be useful in the diagnostics of tuberculous pleuritis [3].

TEST 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 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.

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

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

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

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

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
Tris HCl, pH 8.0	50 mmol/L
4-Aminoantipyrine	2 mmol/L
PNP	0.1 kU/L
XOD	0.2 kU/L
Peroxidase	0.6 kU/L
Reagent 2:	
Tris HCl, pH 4.0	50 mmol/L
Adenosine	10 mmol/L
EHSPT	2 mmol/L

REAGENT PREPARATION

The reagents are ready to use.

REAGENT STABILITY AND STORAGE

Conditions: R1 is light sensitive. Protect from light!
Store in a dark place.
Close immediately after use.
Do not freeze the reagents!
Avoid contamination.

Stability: at 2 – 8 °C up to the expiration date
The reagent should be clear. If turbid, the reagent may have deteriorated.

SAMPLE PREPARATION

Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant. Prompt separation from cells or clot is recommended.
Pleural fluid should be collected in a sterile or heparinized tube resp.
Cerebrospinal fluid (CSF) should be clear and collected in a sterile tube without anticoagulant.

SAMPLE STABILITY AND STORAGE

Serum/plasma [3]**:**	at 2 – 4 °C	1 week
Pleural fluid [6,7,8]:	room temp.	2 hours
	at 2 – 4 °C	2 days
	at -20 °C	2 days
	at -80 °C	up to 2.5 years
Cerebrospinal fluid [9]:	at 25 °C	24 hours
	at 4 °C	7 days
	at -20 °C	3 months

Keep serum/plasma tightly stoppered.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Calibrator	Sample
Reagent 1	900 µL	900 µL	900 µL
Sample or Std./Cal.	-	25 µL	25 µL
Distilled water	25 µL	-	-
Mix. Incubate for 3 min. at 37°C. Then add:			
Reagent 2	450 µL	450 µL	450 µL
Mix. Incubate 5 min. at 37 °C and read A1 against reagent blank. Incubate for exactly 3 min. at 37 °C and read A2 against reagent blank. Calculate: ΔA = (A2 - A1)			

CALCULATION

$$\text{ADA [U/L]} = \frac{\Delta A\ \text{Sample}}{\Delta A\ \text{Calibrator}} \times \text{Conc. Cal [U/L]}$$

REFERENCE RANGE *

Serum [1-4]:	0 – 15 U/L
Pleural fluid [4,5]:	0 – 30 U/L
CSF [4,5]:	0 – 9 U/L

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY / MEASURING RANGE

The test has been developed to determine ADA concentrations within a measuring range from 0.03 – 200 U/L. When values exceed this range, samples should be diluted with NaCl solution (9 g/L) and re-assayed multiplying the result by the dilution factor.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 0.03 U/L

PRECISION (at 37°C)

Within run, n = 30	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	11.1	0.16	1.47
Sample 3	30.7	0.45	1.45

Run to run, n = 30	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	9.63	0.47	4.90
Sample 3	29.6	0.59	2.00

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	4 mg/dL
Bilirubin	30 mg/dL
Hemoglobin	200 mg/dL
Triglycerides	750 mg/dL

CALIBRATION

The assay requires the use of an ADA calibrator. We recommend the Dialab **ADA Calibrator** and 0.9% saline as a zero calibrator.

QUALITY CONTROL

All controls with ADA values determined by this method can be used. We recommend the Dialab **ADA Control Set**.
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Applications for automated systems are available upon request.

WARNINGS AND PRECAUTIONS

1. The reagents contain < 0.1% sodium azide 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.
2. Avoid ingestion and contact with skin and eyes.
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

WASTE MANAGEMENT

Please refer to local requirements.

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