ADENOSINE DEAMINASE (ADA) Multi-Purpose (MPR) Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL2205AA	2 x 20mL	R1	2-8°C
	1 x 20mL	R2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the determination of Adenosine Deaminase (ADA) in serum, plasma, pleural fluid and Cerebrospinal Fluid (C.S.F).

SUMMARY AND EXPLANATION: [1]

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. This enzyme is widely distributed in human tissues particularly 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 tuberculosis effusions. Determination of ADA activity in patient serum may be of value in the diagnosis of liver disease in combination with ALT or γ-GT (GGT) tests. The ADA assay may also be useful in the diagnosis of tuberculosis pleuritis.

PRINCIPLE OF THE TEST: [2]

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-AA) in the presence of peroxidase (POD) to generate quinone dye, the formation of which can be measured spectrophotometrically and is proportional to the level of ADA in the sample.

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

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

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

$$2H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye (max 556nm)}$$

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

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Safety precautions:
This product is not hazardous under EU specifications. Material Safety Data Sheet is available upon request.
- Do not pipette by mouth.
- Exercise the normal precautions required for handling laboratory reagents.
- Solution R1 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
- All specimens used in this test should be considered potentially infectious. Universal precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.
- Do not interchange or mix reagents of different lot numbers.

Components Colour and Appearance:
Reagent 1: Light yellow coloured liquid.
Reagent 2: Clear colourless liquid.
Any significant changes from the above could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Handling precautions:
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:

Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	Ingredients	Concentrations
Reagent 1	Tris	25 mmol/L
	4-AA	4 mmol/L
	PNP	< 0.5 U/mL
	XOD	> 0.2 U/mL
	Peroxidase	0.1 U/mL
Reagent 2	Tris	25 mmol/L
	Adenosine	11 mmol/L
	EHSPT	< 5 mmol/L

REAGENT PREPARATION AND STABILITY:

Reagents are ready to use.
Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, component is stable until expiry date stated on the label. Once opened, reagent is stable for 4 weeks on board the analyser.

TYPE OF SPECIMEN: [1]

Use fresh and non-haemolysed serum or plasma.

It is recommended to follow CLSI procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Serum/Plasma should be separated from cells within 1 hour after collection.
Stability: ADA will be stable in the sample for up to 7 days at 4®C.

TEST PROCEDURE:

Materials required but not supplied:

Description	Catalogue. No.	Description	Catalogue. No.
ADA Calibrator	GL9808	Photometer	N/A
ADA Control Level 1	GL9304	General Laboratory Equipment	N/A
ADA Control Level 2	GL9305		

Assay procedure:
Wavelength: λ: 546 nm
Temperature: 37°C
Optical path: 1 cm light path.

	Blank	Calibrator	Sample
Reagent 1	900 µl	900 µl	900 µl
Sample	----	----	25 µl
Calibrator	----	25 µl	----
Gently mix and Incubate at 37°C Measure the Optical Density (OD1) after 5 minutes.			
Reagent 2	450 µl	450 µl	450 µl
Gently mix and Incubate at 37°C Measure the Optical Density (OD2) after 1 minute.			

Calibration:
Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the laboratory QC Program.

CALCULATION:

$$\text{Concentration} = \frac{\Delta A_{sample} / \text{min}}{\Delta A_{calibrator} / \text{min}} \times \text{Calibrator value}$$

EXPECTED VALUES:

Healthy subjects have an ADA activity in the range of 4-20 U/L, or 66-398 nkat/L.

Each laboratory should establish its own expected values. The ADA results should always be reviewed with the patient's medical examination.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
This assay is linear up to 200 U/L.
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.

Interfering substances:
Results of study are as follows:

Vc: Less than 10% interference up to 20 mg/dL.
Haemoglobin: Less than 10% interference up to 800 mg/dL.
Intralipid: Less than 10% interference up to 1000 mg/dL.

Precision:

Within Run N = 20	Mean *(U/L)*	*SD*	*% CV*	*Between Run N = 20*	Mean *(U/L)*	$\bar{x}$	*(Xmax – Xmin) / $\bar{x}$*
Level 1	29.0	0.17	0.59	Batch 1	28.6	28.6	(28.7-28.6)/28.6*100 = 0.34 %
Level 2	140.4	0.29	0.20	Batch 2	28.6		
				Batch 3	28.7		

BIBLIOGRAPHY:

1. Kobayashi F, Ikeda T, marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. Am. J. Gastroenterol. 88: 266-271 (1993).
2. Kalikan 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(30 389-386 (1999).
3. Burgess LJ, Maritz FJ, Le Roux I, et al. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 50: 672-674 (1995).

SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	STD	Aqueous Standard

CE Mark - Device complies with the Directives 98/79/EC

Storage temperature
Reconstitute with
Expiry Date (Last day of the month)
Manufactured By
Biological risk
Consult Instruction for Use

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