RANDOX

GLUTAMATE DEHYDROGENASE (GLDH)

MANUAL
RX MONZA

INTENDED USE

For the quantitative determination of Glutamate Dehydrogenase (GLDH) in serum. This product is suitable for manual use and on the RX **monza** analyser.

NOT for Clinical Diagnostic Use

Cat. No.		
GL 441/MD	R1a. Buffer/Substrate	1 x 70 ml
8 x 6 ml	R1b. Enzyme/Coenzyme	8 x 6 ml
	R2. α-oxoglutarate	2 x 10 ml

This is an optimized standard method according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie. This procedure measures the non-specific creep reaction.

PRINCIPLE[1]

$$\alpha\text{-oxoglutarate} + NADH + NH_4^+ \xrightarrow{GLDH} glutamate + NAD^+ + H_20$$

SAMPLE

Serum.
Haemolytic and lipaemic sera interfere with the assay.

REAGENT COMPOSITION

Contents		Concentrations in the Test
R1a.	**Buffer/Substrate**	
	Triethanolamine buffer	50 mmol/l, pH 8.0
	Ammonium acetate	100 mmol/l
	EDTA	2.5 mmol/l
R1b.	**Enzyme/Coenzyme**	
	ADP	1.0 mmol/l
	NADH	0.2 mmol/l
	LD	≥ 2 U/ml
R2.	**α-oxoglutarate**	7 mmol/l

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solutions R1a and R2 contain 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.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety data sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1a. Buffer/Substrate
Contents ready for use. Stable up to the expiry date, when stored at +2 to +8°C.

R1b. Enzyme/Coenzyme
Reconstitute the contents of one bottle of Enzyme/ Coenzyme (R1b) with **6 ml** of Buffer/Substrate (R1a). Stable for 1 week at +2 to +8°C.

R2. α-oxoglutarate
Reconstitute the contents of one vial of α-oxoglutarate (R2) with 10 ml of redistilled water. Stable for 8 weeks at +2 to +8°C or 7 days at +15 to +25°C.

NB: If using this assay on an automated system, please refer to procedure sheet for that system as reconstitution instructions maybe different.

MATERIALS PROVIDED

Buffer/Substrate
Enzyme/Coenzyme
α-oxoglutarate

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
Randox Calibration Serum Level 3 (Cat. No. CAL 2351)

PROCEDURE

Select GLDH in the Run Screen and carry out a water blank as instructed.

Pipette into a test tube:	
Sample	0.10 ml
Reagent R1	0.50 ml
Mix and incubate for exactly 5 min at 37°C or 10min at 20-25°C	
Reagent R2	0.02 ml

Mix and aspirate into the RX **monza** immediately.

RX MONZA CALIBRATION

The use of Saline and Randox Calibration Serum Level 3 is recommended for calibration. Calibration is recommended with change in reagent lot or as indicated by quality control procedures.

FOR MANUAL USE

Wavelength:	340 nm (Hg 334 nm or Hg 365 nm)	
Cuvette:	1 cm light path	
Temperature:	25°C	
Measurement:	against air	
Pipette into cuvette:	Macro	Semi Micro
Enzyme/Coenzyme (25°C) (R1)	2.5 ml	1.0 ml
Serum	0.5 ml	0.2 ml
Mix well and let stand at 25°C for 3 minutes, then measure absorbance A1, let stand for exactly 5 min and read absorbance A2 (non-specific creep reaction).		
α-oxoglutarate (R2)	0.1 ml	0.04 ml

Mix well and read absorbance A3, let stand for exactly 5 minutes at 25°C and read absorbance A4.

$$(A3\text{-}A4) - (A1\text{-}A2) = \Delta A / 5 \text{ minutes}$$

MANUAL CALCULATION

To calculate the GLDH activity use the following formulae:

U/l (25°C) = 197 x ΔA 340 nm / 5 min.
U/l (25°C) = 365 x ΔA Hg365 nm / 5 min.
U/l (25°C) = 201 x ΔA Hg334 nm / 5 min.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

NON-SPECIFIC INTERFERENCE

A non-specific interference with the measurement of glutamate dehydrogenase activity has been observed in 60% of sera. At normal glutamate dehydrogenase activity, it amounts to 0.4 U/l on average and seldom exceeds 1 U/l. To take into account the non-specific interference, it is necessary to estimate a serum blank without α-oxoglutarate.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data were obtained using an RX **monza** analyzer running at 37°C.

LINEARITY

The method is linear up to a concentration of 77.3 U/l.
If the sample concentration exceeds this value, dilute the sample 1+4 with 0.9% NaCl solution and re-assay. Multiply the result by 5.

SENSITIVITY

The minimum detectable concentration of Glutamate Dehydrogenase with an acceptable level of precision was determined as 2.90 U/l.

PRECISION

Within run precision

	Level 1	Level 2
Mean (U/l)	13.5	28.3
SD	0.562	0.985
CV(%)	4.16	3.48
n	20	20

Between run precision

	Level 1	Level 2
Mean (U/l)	13.5	28.3
SD	0.764	1.56
CV(%)	5.66	5.53
n	20	20

REFERENCES

1. Schmidt, E. and Schmidt, F.W. In: Method of Enzymatic Analysis, 3rd ed. H.K. Bergmeyer, J. Bergmeyer, and M. Grosse, Eds. Weirheim, Verlag Chemie, 1983, **3:** 216-227.

(S): 17 Jun 19 pl
Rev. 001