

Monoamine Oxidase Assay Kit (MAO)

Method: Colorimetry

Cat .No.	Size	Instrument
GB8030G	2×75 ml	For Hitachi 717
		& ShimadzuCL7200/8000
GS8031G	3×50 ml	For Hitachi917
		& OlympusAU640/400/600
MAO300	4×75 ml	For Hitachi917
		& OlympusAU640/400/600

INTENDED USE

For uantitative determination of MAO activity in patient serum or plasma samples. For use as an valuable detecting the development of liver fibrosis and cirrhosis.

CLINICAL SIGNIFICANCE

The Monoamine Oxidase (MAO) assay kit is intended for quantitative determination of MAO activity in patient serum or plasma samples. Hepatic fibrosis is characterized by strong accumulation of complex connective tissue proteins such as various types of collagens and proteoglycans. MAO is believed to accelerate the rate of cross-linking reaction of collagen fibers^[1]. Elevated levels of serum MAO activity (particularly MAO-B or so-called benzylamine oxidase) are frequently found in patients with hepatic cirrhosis, chronic heart failure, diabetes, and hyperthyroidism^[2]. Therefore, measurement of serum MAO may afford a valuable mean of detecting the development of liver fibrosis and cirrhosis.

ASSAY PRINCIPLE

The reagents of the assay kit are in stable liquid formulation that allows ease of use coupled with enhanced performance characteristics. The MAO assay is based on the enzymatic oxidation of the synthetic substrate benzylamine of MAO to generate benzylaladehyde, ammonia and hydrogen peroxide (H_2O_2) . The Ammonia then reacts with NADH and α -Ketoglutaric acid in the present of GLD to L-Glutamic acid and NAD+, Concomitant with this coenzyme NADH convert to NAD+, the associate change of absorbance at 340 nm can be directly correlated with the MAO activity (U/L).

$$\begin{array}{c} \text{MAO} \\ \text{R•CH-NH2+O}_2\text{+H}_2\text{O} & \longrightarrow \\ \text{R•CHO+NH}_3\text{+H}_2\text{O}_2 \end{array}$$

GLDH $NH_3 + \alpha$ - Ketoglutaric acid + NADH \longrightarrow L-Glutamic acid + H_2 O + NAD^+

REAGENT COMPOSITON

Contents	Concentration
Buffer	150 mmol/L
benzylamine	20 mmol/L
α- Ketoglutaric acid	10 mmol/L
NADH	0.25 mmol/L
Detergent	1%
Preservative	2 g/L
Stabilizer	2 g/L
Glutamate Dehydrogenase	1 KU/L
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STABILITY AND PREPARATION OF REAGENTS

Reagents are ready to use.

Stable up to the expiry date when stored at 2-8℃.

The MAO assay reagents are stable for 28 days after opening and kept at 2-8 $^{\circ}\mathrm{C}$.

MATERIALS NEEDED BUT NOT PROVIDED

MAO control is not included in the kit and need to be ordered separately. MAO control should be reconstituted with distilled water. The reconstituted MAO control is stable for 1 week at 4°C.

SAMPLE COLLECTION AND PREPARATION

Use fresh and non-haemolyzed serum or plasma for MAO test.

ASSAY PROCEDURE

Test Procedure for Analyzers (HITACHI 7170/917)

Incubation temp: 37° C Assay Mode: 2 Point Rate

Wave Length (main/sub): 340 nm / 410 nm

Sample: 18 μl Reagent: : 180 μl



- 1. Mix 18 µl sample with 180 µl R1 and incubate at 37 $^{\circ}$ C for 4 minutes.
- Read initial absorbance and start timer simultaneously, read again after 2 minutes.
- 3. Calculate absorbance change per minute ($\Delta A/min$).

Calculation

MAO (U/L) = A/min K ($nkat/L=U/L \times 16.7$)

 $K = (10^3 \times Vt) / (\epsilon \times Vs \times L)$

ε: NADH mM extinction coefficient at 340nm (6.3)

Vt: Total reaction volume (ml)

St: Sample volume (ml)

L: length of light path (cm)

K factor varies according to specific instruments.

QUALITY CONTROL

For quality control, use GQ-MAO as quality control sera, values should fall within a specific 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 reaction temperature.
- 3. Check expiration date of kit and contents.
- Check the quality of the water used for reagents reconstitution.

EXPECTED VALUE

Serum: ≤12 U/L.

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

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SPECIFIC PERFORMANCE CHARACTERISTICS

The method is linear up to 100 U/L. Sample above this concentration should be diluted with 0.9% NaCl and reassay. Multiply the result by dilution factor.

PRECISION

Precision studies conducted by using one serum sample assayed at least ten times has a precision of 3.10% of intra CV and 5.00% of inter CV.

INTERFERENCE

The following analyze concentrations were not found to affect the assay:

Ascorbic acid: 10 mg/dl 40 mg/dl Heparin: Bilirubin: 50 ma/dl Hemoglobin: 300 mg/dl Ammonia: 50 mg/dl

CORRELATION

This method (y) was compared with another commercially available method and the following linear regression equation obtained:

 $y = 0.853 \times -0.543$, $R^2 = 0.926$

SAFETY PRECAUTIONS AND WARNINGS

- For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
- solution R1a 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.

- 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.
- 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.

REFERENCES

- Hare MLC(1928)Tyramine oxidase.I.A new enzyme system in liver. Biochem J 22: 968Y979
- Yu P, Boulton A(1987)."Irreversible inhibition of monoamine oxidase by some components of cigarette smoke". Life Sci 41(6):675-82. PMID 3613836

INDEX OF SYMBOLS



Manufacture

Catalogue Number

Lot number

Date of manufacture



Use by(Expiration date)



For In-Vitro Diagnostic use only



Stored at 2-8°C



REP

Attention:See instruction for use

Authorized Representative in the **European Company**

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