

SGOT REAGENT KIT

(Mod. IFCC Method) For photometric determination of SGPT in serum For In vitro diagnostics only **Ref no.** SGOT25

SGOT25 SGOT125

Summary

SGOT (AST) is an enzyme found mainly in heart muscle, skeletal muscle, liver cells and kidneys. Injury to these tissues results in the release of enzyme in blood. Elevated levels are found in myocardial infarction, cardiac operations, cirrhosis, Hepatitis, acute pancreatitis, acute renal disease, primary muscle disease. Decreased levels may be found in pregnancy, Beri Beri and diabetic ketoacidosis.

Principle

SGOT (AST) catalyses the transfer of amino group between L-Aspartate & α -ketoglutarate to form Oxalacetate & L-Glutamate. The Oxalacetate formed reacts with NADH in the presence of malate dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT activity in the sample.

L-Aspartate + α -ketoglutarate $\stackrel{\text{sGOT}}{\longrightarrow}$ L-Glutamate + Oxalacetate

Oxalacetate +NADH+H⁺ MDH

L-malate + NAD⁺

Kit Contents

| | Kit size | 25ml | 125ml | |
|--|----------|--------|---------|--|
| | Ref no. | SGOT25 | SGOT125 | |
| | SGOT –R1 | 1 | 2 | |
| | SGOT-R2 | 1 | 1 | |
| | IFU | 1 | 1 | |

Material required not provided

Test tubes, yellow tips, blue tips General laboratory equipment

Storage & Stability of the Reagents

- 1. The reagents are stable till the date of expiry, when stored at 2⁰-8⁰ C, protect from light &contamination is avoided.
- 2. Do not freeze the reagents.
- 3. Ensure the reagents shelf life is valid.
- 4. Do not use reagent if:
 - The initial absorbance at 340nm is below 0.800.
 - The reagent fails to meet stated parameters of performance.

Reagent preparation

Mix, 4 parts of reagent 1 & 1 part of reagent 2 = working reagent.

The stability of the working reagent is

7 days at $15^{\circ}-25^{\circ}C$ &

4 Weeks at 2^{0} - 8^{0} C.

Alternatively 0.8ml of R1& 0.2ml R2 may also be used instead of 1ml of the working reagent directly during the assay.

The working reagent should have an absorbance above 1.0 against distilled water at 340nm.

Discard the reagent if the absorbance is below 1.0

Reagent composition

| Reagent 1 | Tris buffer Ph 7.8 | | 6gm/L |
|---------------------------------|--------------------|----------|----------|
| | L-Asparta | ate | 30.5gm/L |
| LDH(lactate dehydrogenase)4k U/ | | e)4k U/L | |
| | MDH | | 700 U/L |
| Reagent 2 | α-ketogl | utarate | 3.4gm/L |
| | NADH | | 1.1 gm/L |
| | | | Ũ |

Specimen

Serum

Specimen collection

1. Fresh, clear, non-hemolyzed serum is recommended. Red cells contain SGOT which can give falsely elevated results.

Storage & Stability of the Specimen

SGOT in serum is reported stable for ten days when refrigerated (2-8°C), two weeks when frozen (-20°C), and four days when stored at room temperature (15- 30° C).

Warning & Precautions

- 1. Keep out of reach children. In case of contact with eyes, rinse immediately with plenty of water &seek medical advice.
- 2. Take off immediately all contaminated clothing.
- 3. Wear suitable gloves and eye /face protection.
- 4. Always use safety pipettes to pull the reagents into a pipette.
- 5. Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.
- 6. Perform the test according to the current "Good Laboratory Practice"(GLP) guidelines.



SWEMED DIAGNOSTICS

7. The reagents contain sodium azide (0.95g/L) as preservative. Do not swallow. Avoid contact with skin and mucous membrane.

Assay procedure

| Wave length | : 340 nm | |
|--------------|-------------------------|-------------|
| Temperature | : 37° c | |
| Light path | : 10 mm | |
| Pipette into | Macro | Semi-Micro |
| cuvettes | | |
| Reagent | 800μ l +200 μ l | 400µ1+100µ1 |
| (R1+R2) | | |
| Sample | 100µ1 | 50µ1 |
| _ | | |

Mix well & read the initial absorbance A_0 after 1minute and repeat the absorbance reading after every 1, 2, & 3minutes. Calculate the mean absorbance change per minute (ΔA /min).

Calculation

SGOT (U/L) = $\Delta A/\min \times 1746$

Performance Characteristics Measuring range

The test has been developed determine SGOT activities which correspond to a maximal A/min of 0.16 at 340nm. If such value is exceeded the sample should be diluted 1+9 with NaCl solution (9g/l) & result multiplied by 10.

Linearity

The linearity is 300U/L

Interferences

Bilirubin to at least 18 mg/dl, and hemoglobin to at least 300 mg/dl, have been found to have a negligible effect on this procedure.

Reference range

| Women | <31 U/L |
|-------|---------|
| Men | <32 U/L |

Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Quick References

| - | |
|---------------|---------------|
| Parameter | SGOT |
| Mode | Kinetic |
| Wavelength | 340nm |
| Unit | IU/L |
| Temperature | 37°C |
| Factor | 1746 |
| Reaction slop | pe Decreasing |
| Reagent volu | $1000 \mu l$ |
| Sample volu | me 100μ l |
| Reaction tim | e 180sec |
| Delay time | 60sec |
| Delta time | 60sec |
| Blanking | Water blank |
| linearity | 300 U/L |
| linearity | 300 U/L |

Literature

- 1. Clin. Chem. ACTA 105 (1980) S. 147 172.
- Synopsis Der Leberkrankheiten: H. Wallhofer, E. Schmidt U.F.W. Schmidt, G. Thieme Verlag stuttgart 1974.
- 3. Thefeld W.ET. AL. DTSCH. MED. WSCHR. 99 (1974) 343.
- 4. Bergmeyer, H.U., et.al. (1986) J. Clin. Chem. Clin. Biochem. 24: 497.

Note on symbols and marks

