

For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

乙酰乙酸检测试剂盒

Acetoacetate Assay Kit



Do not eat Store at -20° C & in the dark

Cat.No. MAA6558

Size : 100 tests

Technical literature is available at: www.mesgenbio.com

E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com

Description

Acetoacetate (AAT), a β -ketoacid, is one of the three ketone bodies and is formed via condensation of two molecules of acetyl-CoA in liver mitochondria. AAT can be enzymatically reduced to 3- β -hydroxybutyrate (β -HB), or decarboxylated producing acetone ((CH₃)₂CO). Ketone bodies [β -HB: 78%; AAT: 20% & (CH₃)₂CO: 2%] are mainly used as an alternative energy source when glucose cannot be delivered to the system. Excessive concentration of ketone bodies (ketoacidosis) is observed in patients with Type I diabetes, severe starvation or alcoholism. Simple, direct and automation-ready procedures for measuring acetoacetate (AAT) are very desirable. MesGen Biotech' Acetoacetate assay is based on a reaction with Sodium Nitroprusside forming a product with absorbance at 550 nm.

Applications

Direct assay of acetoacetate in serum, plasma, urine and other biological samples.

Key Features

Sensitive and accurate. Linear detection range of 0.005 to 1 mM for acetoacetate in 96-well plate assay.

Note: Considering the influence of acetone interfere, if the AAT concentration in sample is >1 mM, please dilute sample in H₂O and repeat this assay.

Convenient. The procedure involves adding a single working reagent and reading the optical density at room temperature in kinetic mode.

High-throughput. Can be automated as a high-throughput 96-well plate assay for thousands of samples per day.

Kit contents (100 tests in 96-Well plates)

Reaction Buffer : 15 mL

Reaction Reagent: 1 tube (Dried)

Lithium acetoacetate Standard 100 mM: 1 mL

Procedures

Samples: serum and plasma samples should be non-hemolyzed and assayed immediately. If not assayed, samples can be stored at -80°C for up to 30 days.

Working Reagent preparation: bring all reagents to room temperature prior to assay. Reconstitute the Reaction Reagent with 15 mL Reaction Buffer. Oscillated until the buffer was dissolve thoroughly. Unused Working Reagent is stable for one month when stored frozen at -80°C.

- Standards. Prepare 1 mM AAT standard by mixing 2 μ L AAT standard with 198 μ L distilled H₂O. Prepare standard dilutions as follows:

No.	Standard + H ₂ O	AAT (mM)
1	100 μ L + 0 μ L	1
2	80 μ L + 20 μ L	0.8
3	50 μ L + 50 μ L	0.5
4	30 μ L + 70 μ L	0.3
5	10 μ L + 90 μ L	0.1
6	5 μ L + 95 μ L	0.05
7	1 μ L + 99 μ L	0.01
8	0 μ L + 100 μ L	0

- Samples. Transfer 100 μ L of each sample and Standard to separate wells.
- Reaction. Add 100 μ L Working Reagent to each well. Gently tap plate to mix. Incubating at room temperature for 5 minutes and protected from the light, read OD_{550nm}. Calculate the acetoacetate concentration from the OD values.

Calculations

Subtract the Blank value from the standard values and plot ΔOD against the standard concentrations. Determine the slope of the line and calculate the acetoacetate concentration of the samples as follows:

$$[AAT] = \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{\text{Slope (mM}^{-1})} \times n \text{ (mM)}$$

Storage conditions

The kit is shipped at dry ice. Store all reagents at -20°C .

Shelf life

6 months after receipt, the Working Reagent of reconstitution should be stored at -80°C and used within 1 month.

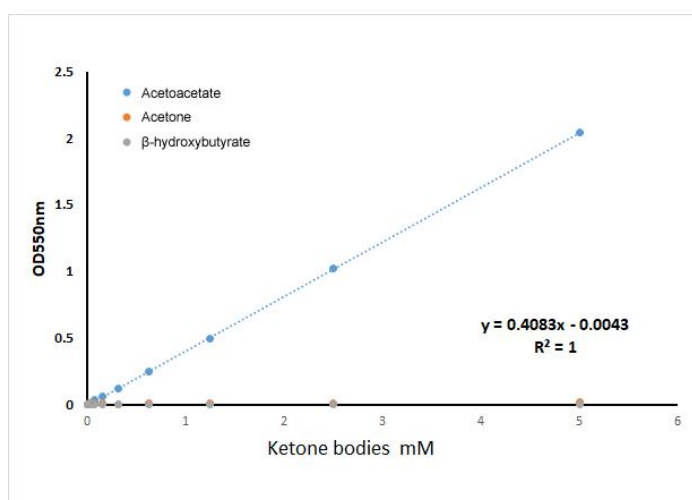


Fig.1 Standard Curve for equimolar amounts of Acetoacetate, β -HB and Acetone (RT, 5 min.). The other two ketone bodies β -hydroxybutyrate or Acetone are not detected obviously in the assay due to much lower sensitivity in the linear detection range of 0.005 to 1 mM.

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