Acetoacetate Assay Kit

Product Datasheet | SKU BA0265

Description

Acetoacetate is one of three ketone bodies produced in the liver, mainly from the oxidation of fatty acids, and is normally present at low concentrations in urine and blood. Increased ketone concentrations in the blood may lead to metabolic acidosis, which has been associated with diabetes, childhood hypoglycaemia, growth hormone deficiency, alcohol or salicylate intoxication and inborn errors of metabolism. Acetoacetate is an unstable ketone body that spontaneously decarboxylates to release carbon dioxide and acetone. This assay provides a simple, direct and automation-ready procedure for measuring acetoacetate, based on a reaction with sodium nitroprusside that forms a product with absorbance at 550 nm.

Specifications

Detection Method	Colorimetric (OD 550 nm)
Detection Range	0.012 to 1 mM (1.2 - 100 nmole/well)
Sample Type	Serum, plasma, urine and other biological samples
Species Reactivity	All
Assay Time	30 minutes (kinetic read)
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	6 months
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
AAT Buffer	10 mL	-20°C
AAT Reagent	1 mL	-20°C
AAT Standard	Dried	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0265	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Bring all reagents to room temperature prior to assay and reconstitute the AAT Standard tube with 100 µL distilled water (final 100 mM); unused standard is stable for two months when stored frozen at -20°C.
2. Prepare a 1 mM AAT working standard by mixing 2 µL AAT standard with 198 µL distilled water, then prepare standard dilutions of 1.0, 0.6, 0.3 and 0 mM in a final volume of 100 µL.
3. Transfer 10-100 µL of each sample to separate wells and bring to a final volume of 100 µL with distilled water.
4. Prepare Working Reagent by mixing 95 µL AAT Buffer and 10 µL AAT Reagent for each well, then add 100 µL Working Reagent to each well and gently tap the plate to mix.
5. Incubate at room temperature protected from light and read OD550nm in kinetic mode for 30 minutes, calculating the acetoacetate concentration from the OD values at maximum absorbance.

6. If the levels of acetoacetate are expected to be low or if matrix effects are present, use an internal standard by running two reactions per sample: sample plus internal standard (20 µL of 1 mM AAT standard added to 80 µL sample) and sample alone (20 µL distilled water added to 80 µL sample).

Standard Preparation

No	Standard + H2O	Vol (µL)	AAT (mM)
1	100µL + 0µL	100	1.0
2	60µL + 40µL	100	0.6
3	30µL + 70µL	100	0.3
4	0µL + 100µL	100	0

Calculation

Subtract the Blank value (standard #4) from the standard values and plot delta-OD against the standard concentrations to determine the slope. [AAT] = (ODSAMPLE - ODBLANK) / Slope (mM-1) x n (mM), where n is the sample dilution factor. If an internal standard is used: [AAT] = (ODSAMPLE - ODBLANK) / (ODSTAND - ODSAMPLE) x 0.25 x n (mM), where ODSTAND is the OD of the sample plus internal standard.

Notes

- Serum and plasma samples should be non-hemolysed and assayed immediately; if not assayed, samples can be stored at -80°C for up to 30 days.
- If the calculated acetoacetate concentration is greater than 1 mM, dilute the sample in water, repeat the assay and multiply the result by the dilution factor.
- The AAT standard should be used within 2 months of reconstitution.
- Normal precautions for laboratory reagents should be exercised while using the reagents.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.