QuantiChrom™ Acetoacetate Assay Kit (DAAT-100)

Quantitative Colorimetric Determination of Acetoacetate

DESCRIPTION

ACETOACETATE is one of three ketone bodies produced in the liver mainly from oxidation of fatty acids and are 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 hypoglycemia, 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.

Simple, direct and automation-ready procedures for measuring acetoacetate (AAT) are very desirable. BioAssay Systems' QuantiChrom™ 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 0.012 to 1 mM (1.2 – 100 nmole/well) for acetoacetate in 96-well plate assay.

Convenient. No sample pretreatment needed. 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)

AAT Buffer: 10 mL
AAT Reagent: 1 mL
AAT Standard: Dried

Storage conditions. The kit is shipped at room temperature. Store all reagents at -20°C. Shelf life: 6 months after receipt, the AAT standard should be used within 2 months of reconstitution.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

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.

Reagent preparation: bring all reagents to room temperature prior to assay. Reconstitute the AAT Standard tube with 100 µL dH_2O (final 100 mM). Unused AAT Standard is stable for two months when stored frozen at -20°C.

1. *Standards.* Prepare 1 mM AAT standard by mixing 2 µL AAT standard with 198 µL distilled H_2O. Prepare standard dilutions as follows:

No	Standard + H_2O	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

2. *Samples.* Transfer 10-100 µL of each sample to separate wells and bring to a final volume of 100 µL with dH_2O.
3. *Reaction.* Prepare Working Reagent by mixing 95 µL AAT Buffer and 10 µL of AAT Reagent for each well.

   Add 100 µL Working Reagent to each well. Gently tap plate to mix.
4. Incubating at room temperature and protected from the light, read OD_{550nm} in Kinetic mode for 30 minutes. Calculate the acetoacetate concentration from the OD values at the maximum absorbance.

Note: if the calculated [AAT] is >1 mM, dilute sample in H_2O and repeat this assay. Multiply the results by the dilution factor. If the levels of acetoacetate are expected to be low or if there will be matrix effects, the inclusion of an internal standard is recommended.

Samples requiring an internal standard will need two separate reactions: 1) Sample plus Internal Standard, and 2) Sample alone. For the sample plus standard well, add 20 µL of 1 mM AAT Standard to 80 µL sample. For the sample well, add 20 µL dH_2O to 80 µL sample. Proceed with other steps of the procedure (standards and reaction) as with the normal assay.

CALCULATIONS

Subtract the Blank value (#4) 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:

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

If an internal standard is used, the sample acetoacetate concentration is calculated as follows:

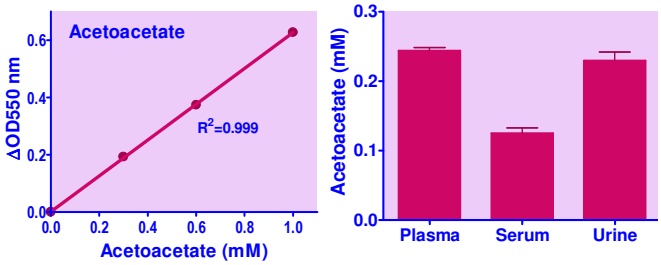
$$[AAT] = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STAND} - OD_{SAMPLE}} \times 0.25 \times n\ (mM)$$

Where OD_{SAMPLE}, OD_{BLANK}, and OD_{STAND} are the optical density reading of the Sample, Blank and Sample plus Internal Standard, respectively. *n* is the sample dilution factor.

Notes: The volume of the internal standard is a fifth of the total sample volume; thus, the sample to standard ratio is multiplied by 0.25 mM to account for the dilution factor associated with the standard.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting (multi-channel) devices. Clear flat-bottom 96-well plates (e.g. Corning Costar) and plate reader.



Standard Curve of Acetoacetate (AAT) (left). Acetoacetate levels from human plasma, serum, and urine using an internal standard (right).

REFERENCES

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