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Acetoacetate Colorimetric Assay Kit

rev. 09/13

(Catalog # K650-100; 100 assays; Store at -20°C)

I. Introduction:

Acetoacetate (AcAc), a β-ketoacid, is one of the three ketone bodies and is formed via condensation of two molecules of acetyl-CoA in liver mitochondria. AcAc can be enzymatically reduced to 3-β-hydroxybutyrate (β-HB), or decarboxylated producing acetone $(CH_3)_2CO$). Ketone bodies (βHB: 78%; AcAc: 20% & $CH_3)_2CO$: 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. Traditionally, AcAc levels have been qualitatively detected using dipsticks that use sodium nitroferricyanide as a chromophore. BioVision's Acetoacetate Assay Kit has adapted that principle with a modification that provides a sensitive method to quantitate endogenous levels of AcAc in human blood, and urine. In this non-enzymatic assay, AcAc reacts with a substrate to generate a colored product that can be measured at 550 nm. The reaction is specific for AcAc and does not detect 3-β-hydroxybutyrate. The assay kit can detect samples containing acetoacetate as low as 25 µM.

Acetoacetate + Substrate ⟶ Absorbance (OD 550 nm)

II. Application:

- Measurement of acetoacetate in various biological samples
- Analysis of acetoacetate in pathological conditions such as Type I diabetes, alcoholism, etc.

III. Sample Type:

- Serum, plasma, blood, urine & other body fluids

IV. Kit Contents:

Components	K650-100	Cap Code	Part Number
Acetoacetate Assay Buffer	15 ml	WM	K650-100-1
Acetoacetate Standard (Lyophilized)	1 vial	Yellow	K650-100-2
Acetoacetate Substrate	1 ml	Amber	K650-100-3

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage and Handling:

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:

- **Acetoacetate Assay Buffer:** Warm Acetoacetate Assay Buffer to room temperature. A small amount of precipitation will typically be observed. Vortex the bottle to dissolve the precipitate before use.
- **Acetoacetate Standard:** Reconstitute with 100 µl ddH_2O to generate 100 mM solution. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.
- **Acetoacetate Substrate:** Acetoacetate Substrate is light sensitive. Minimize the exposure to light. Aliquot & store at 4°C. Keep at room temperature while in use.

VIII. Acetoacetate Assay Protocol:

1. **Sample Preparation:** Acetoacetate is extremely unstable; we recommend processing and analyzing samples immediately after collection. If immediate assay is not possible, store samples up to 4 weeks at -80°C. Add 10-100 µl test samples into the desired well(s) in a 96-well plate. Adjust the volume to 110 µl/well with ddH_2O. For samples having high background such as urea, prepare parallel sample well(s) as the background control.

   Notes:

   a. Acetoacetate concentration can vary over a wide range from 40 to 8400 µM. We suggest using different volumes of your sample to ensure readings are within the Standard Curve range.

   b. Endogenous enzyme activity may cause loss of acetoacetate. To remove endogenous enzymes, use 10 kDa Spin Column (Cat. 1997) & centrifuge samples at 10,000 x g for 10 min. at 4°C. Use collected ultrafiltrate as assay sample.

   c. Endogenous compounds may interfere with the reaction. To ensure accurate determination of acetoacetate in the test samples or for samples having low concentration of acetoacetate, we recommend spiking samples with a known amount of Acetoacetate Standard (30 nmol).

2. **Standard Curve Preparation:** Dilute 100 mM AcAc Standard to 10 mM by adding 10 µl of AcAc Standard to 90 µl of ddH_2O. Mix well. Add 0, 2, 4, 6, 8 & 10 µl of diluted Standard into a series of wells in a 96-well plate to generate 0, 20, 40, 60, 80 & 100 nmol/well of Acetoacetate Standard. Adjust the volume to 110 µl/well with ddH_2O.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 90 µl Mix containing:

	Reaction Mix	* Background Control Mix
Acetoacetate Assay Buffer	80 µl	90 µl
Acetoacetate Substrate	10 µl	----

Add 90 µl of the reaction mix to each well containing the Standards, and test samples. Mix.
* For samples having high background, add 90 µl of Background Control Mix to the sample background control well(s). Mix well.

4. **Measurement:** Incubate the plate at either 25°C for 10-15 min. or 4°C for 80-110 min. Protect the plate from light. Measure the absorbance (OD 550 nm) in a kinetic mode.

Notes:

a. Incubation time depends on the amount of endogenous Acetoacetate levels in the samples and selected temperature. The reaction product is unstable. We recommend measuring the OD in kinetic mode, and monitoring the maximum absorbance value for 100 nmol Acetoacetate Standard.

b. The stability of the final product can be significantly extended if the plate is incubated at lower temperature. Additionally, the OD_{550} values are enhanced.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Acetoacetate Standard Curve. If sample background control reading is significant then subtract the sample background control reading from sample reading. For unspiked samples, apply the corrected OD to the Acetoacetate Standard Curve to get B nmol of acetoacetate during the reaction time.

Sample Acetoacetate concentration (C) = B/V X D nmol/µl = µmol/ml or mM

Where: **B** is the amount of acetoacetate in the sample well (nmol)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Acetoacetate amount in sample well (B) = $\left(\frac{(OD_{sample\ (corrected)})}{(OD_{sample\ +AcAc\ Std(corrected)})-(OD_{sample(corrected)})}\right) * AcAc\ Spike\ (\text{nmol})$

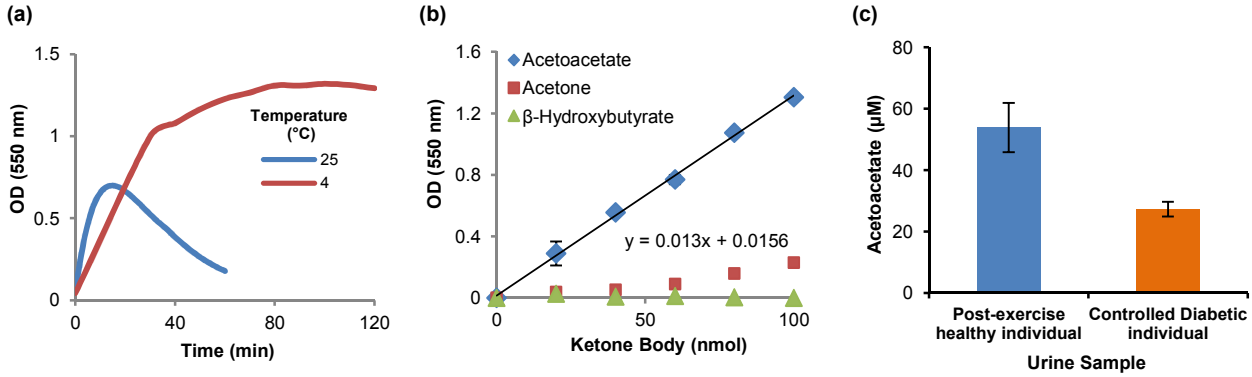

Figure: a) Time course of color development at 4°C and 25°C (100 nmol Acetoacetate). b) Standard Curve for equimolar amounts of Acetoacetate, β-HB and Acetone (4°C, 100 min.). The other two ketone bodies β-hydroxybutyrate or Acetone are not detected in the assay due to much lower sensitivity (β-HB) and much lower concentration (Acetone). c) Measurement of Acetoacetate in urine. Samples were deproteinized by spin filtering (Cat # 1997). 100 µl of undiluted urine was spiked with known amounts of AcAc (30 nmol). Assays were performed following the kit protocol.

IX. RELATED PRODUCTS:

β-Hydroxybutyrate (β-HB) Colorimetric Assay Kit (K632-100)

PicoProbe™ β-Hydroxybutyrate Fluorometric Assay Kit (K651-100)

FOR RESEARCH USE ONLY! Not to be used on humans.