ab180875

Acetoacetate Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of endogenous Acetoacetate in various samples.

This product is for research use only and is not intended for diagnostic use.

Version 5a Last Updated: 1 July 2025

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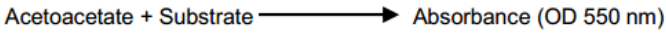
1. Overview

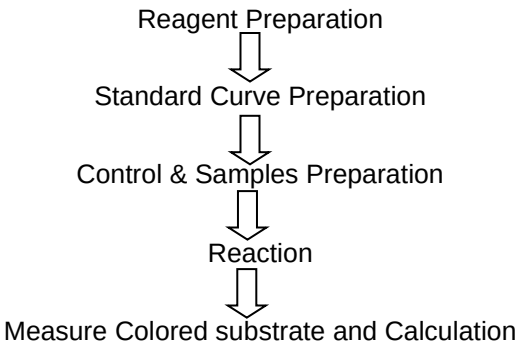
Acetoacetate (AcAc), a β-ketoacid, is one of the three ketone bodies and is formed via condensation of two molecules of acetyl-CoA in liver mitochondria. AcAc can be enzymatically reduced to 3-β-hydroxybutyrate (β-HB), or decarboxylated producing acetone $(CH_3)_2CO$. Ketone bodies (βHB: 78%; AcAc: 20% & $CH_3)_2CO$: 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.

Abcam's Acetoacetate Assay Kit (Colorimetric) (ab180875) has adapted that principle with a modification that provides a sensitive method to quantitate endogenous levels of AcAc in human 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.

Figure 1: Assay Procedure



2. Protocol Summary



3. Kits Components

Item	Quantity
Acetoacetate Assay Buffer	15 mL
Acetoacetate Standard	1 vial
Acetoacetate Substrate	1 mL

4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- Distilled water (dH_2O) or MilliQ
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Centrifuge with cooling option

6. Reagents Preparation

1. **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.

2. **Acetoacetate Standard:**
   Reconstitute with 100 µL dH_2O to generate 100 mM solution. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.

3. **Acetoacetate Substrate:**
   Acetoacetate Substrate is light sensitive. Minimize the exposure to light. Aliquot & store at -20°C. Keep at room temperature while in use.

7. 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.

NOTE:

- *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.*
- *Endogenous enzyme activity may cause loss of acetoacetate. To remove endogenous enzymes, use 10 kD Spin Column (ab93349) and centrifuge samples at 10,000 x g for 10 min. at 4°C. Use collected ultrafiltrate as assay sample.*
- *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 Acetoacetate Standard to 10 mM by adding 10 µL of Acetoacetate Standard to 90 µL of ddH2O. 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 ddH2O.

3. Reaction Mix:

Prepare enough Reaction Mix 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.*

Mix enough reagents for the number of assays (samples and standards) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

Reaction Mix	
Acetoacetate Assay Buffer	80 µL x (Nb samples + Standards +1)
Acetoacetate Substrate	10 µL x (Nb samples + Standards +1)

4. Measurement

a) Incubate the plate at either 25°C for 10-15 min. or 4°C for 80-110 min. Protect the plate from light.

b) Measure the absorbance (OD 550 nm) in a kinetic mode.

NOTE:

- *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.*
- *The stability of the final product can be significantly extended if the plate is incubated at lower temperature. Additionally, the OD550 values are enhanced.*

8. Data Analysis

Calculations:

a) Correct background by subtracting the value derived from the zero standard from all sample readings.

b) 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.

c) 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

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

Acetoacetate amount in sample well (B) =

$$\left(\frac{ODsample(corrected)}{(ODsample+AcAc\ Std(corrected)-(ODsample(corrected)}\right) * AcAc\ Spike(nmol)$$

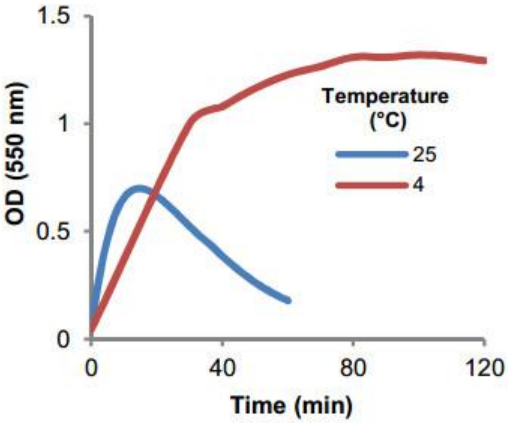


Figure 1. Time course of color development at 4°C and 25°C (100 nmol Acetoacetate).

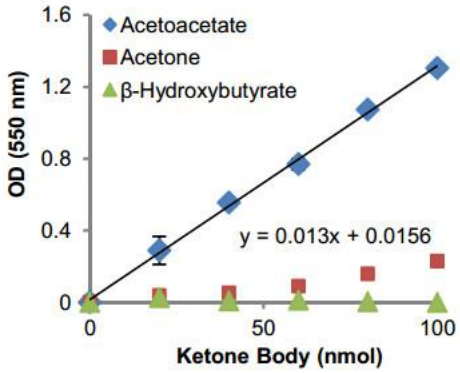


Figure 2. Standard Curve for equimolar amounts of Acetoacetate, β HB and Acetone (4°C, 100 min.). The other two ketone bodies β-hydroxybutyrate or Acetone are not detected in the assay due to much lower sensitivity (β-HB) and much lower concentration (Acetone).

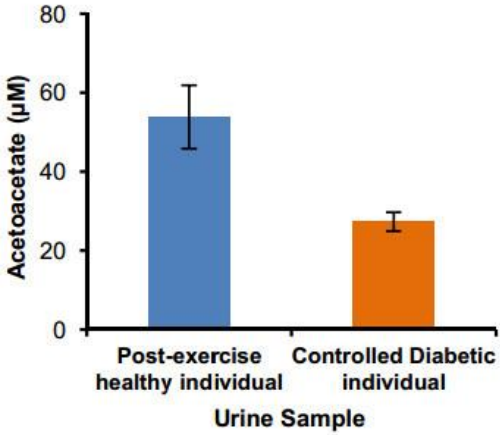


Figure 3. Measurement of Acetoacetate in urine. Samples were deproteinized by spin filtering. 100 µL of undiluted urine was spiked with known amounts of AcAc (30 nmol). Assays were performed following the kit protocol.

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)