AB318308 – Ketone Body Assay Kit (Colorimetric)

For the measurement of ketone bodies in various samples.

For overview, typical data and additional information please visit: www.abcam.com/ab318308
(use www.abcam.cn/ab318308 for China, or www.abcam.co.jp/ab318308 for Japan)

Storage and Stability: Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt if components have not been reconstituted.
Reconstituted components are stable for 2 months.
Aliquot components in working volumes before storing at the recommended temperature.
Avoid repeated freeze-thaws of reagents.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
β-HB Assay Buffer	25 mL	-20 °C	4 °C
β-HB Reagent	1 vial	-20 °C	-20 °C
β-HB Standard	1 vial	-20 °C	-20 °C
β-HB Enzyme Mix	2 vials	-20 °C	-20 °C
β-HB DH Diluent	1mL	-20 °C	4 °C
Acetoacetate Assay Buffer II	25 mL	-20 °C	4 °C
Acetoacetate Reagent	1vial	-20 °C	-20 °C
Acetoacetate Standard	1vial	-20 °C	-20 °C
Developer Solution II	1vial	-20 °C	-20 °C

Materials Required, Not Supplied
Microplate reader capable of measuring absorbance
Clear 96 well plate with flat bottom
dH_2O
10 kDa MWCO spin filters (ab93349) (optional)

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening.

1. **β-HB Assay Buffer and Acetoacetate Assay Buffer II:** Ready to use. Equilibrate to room temperature (RT) before use.
2. **β-HB Reagent and Acetoacetate Reagent:** Reconstitute each vial with 330 µL dH2O. Divide into aliquots and store at -20 °C. Avoid multiple freeze/thaw cycles. Keep on ice while in use.
3. **β-HB Standard:** Reconstitute the vial with 100 µL dH2O to generate a 10 mM solution. Divide into aliquots and store at -20 °C. Keep on ice while in use.
4. **β-HB Enzyme Mix:** Reconstitute each vial with cold 220 µL β-HB DH Diluent. Divide into aliquots and store at -20 °C. Keep on ice during use. Avoid repeated freeze-thaw cycles. Stable for at least two months.
5. **β-HB DH Diluent:** Thaw on ice and store at 4 °C.
6. **Acetoacetate Standard:** Reconstitute the vial with 100 µL dH2O to generate a 100 mM solution. Divide into aliquots and store at -20 °C. Use within two months. Keep on ice while in use.
7. **Developer Solution II:** Reconstitute the vial with 1.2 mL of dH2O. Pipette up and down several times to completely dissolve the pellet into solution (don't vortex). Divide into aliquots and store at -20 °C, protected from light. Use within 2 months.

Sample Preparation
ΔNOTE For Unknown Samples, we suggest testing several doses of the sample to make sure the readings are within the Standard Curve ranges.
Serum and Plasma Samples:
ΔNOTE: Serum and plasma samples should be non-hemolyzed and processed immediately after collection. Acetoacetate is unstable and will rapidly degrade to acetone and CO2 at room temperature. We recommend analyzing serum/plasma samples promptly after collection. If immediate assay is not possible, store samples at -80 °C (for up to 4 weeks).

1. Centrifuge serum/plasma at 14,000 x g and 4 °C for 10 min.
2. Transfer the supernatant to a new microfuge tube. Keep all samples on ice during the assay.

ΔNOTE: To eliminate potential sources of enzymatic interference in plasma/serum, samples can be deproteinized using 10 kDa MWCO spin filters (ab93349). Centrifuge samples through 10 kDa MWCO spin filter at 10,000 x g and 4 °C for 10 min and collect the filtrate for use directly in the assay.

3. Prepare two wells for each sample to be tested: a Sample (S) well and a parallel well for the same sample that will be used as a Sample Background Control (BC). Add 2-50 µL of the sample supernatant into parallel wells in a clear 96-well plate (with flat bottom) for each assay to be performed.

ΔNOTE: Incubation times vary for these two assays so plan plate experiment accordingly, either by grouping the assays on the plate or using separate plates for the two assay types.

4. Adjust the volume of all wells to 50 µL/well with Hydroxybutyrate Assay Buffer for the β-Hydroxybutyrate (β-HB) Assay or Acetoacetate Assay Buffer II for the Acetoacetate assay.

β-Hydroxybutyrate (β-HB) Assay:
β-HB Standard Preparation
Always prepare a fresh set of standards for every use & discard working standard dilutions after use as they do not store well.

1. Prepare a 1 mM β-HB Standard working solution by mixing 10 µL of 10 mM β-HB Standard with 90 µL of dH_2O.
2. Add 0, 2, 4, 6, 8, 10, 15 and 20 µl of the diluted 1 mM β-HB Standard into a series of wells.
3. Adjust the volume to 50 µL/well with β-HB Assay Buffer to generate 0, 2, 4, 6, 8, 10, 15 and 20 nmol/well of β-HB Standard.

ΔNOTE: To improve accuracy and reduce CVs, the standard curve may be prepared in duplicate, in a microplate or microcentrifuge tubes using the table below. Each standard mix has enough volume to set up duplicate readings (2 x 50 µL).

Standard #	1 mM β-HB Standard (µL)	β-HB Assay Buffer (µL)	Final volume standard in well (µL)	β-HB in well (nmol)
1	0	125	50	0
2	5	120	50	2
3	10	115	50	4
4	15	110	50	6
5	20	105	50	8
6	25	100	50	10
7	45	105	50	15
8	50	75	50	20

β-HB Assay Procedure

ΔNOTE: Equilibrate all materials and prepared reagents to RT just prior to use and gently agitate. Assay all standards, controls and samples in duplicate.

1. Make enough β-HB Reaction Mix and Sample Background Mix for the number of assays to be performed, 50 µl per reaction as shown below. Remember to account for the β-HB Standard Curve wells when calculating the amount of Reaction Mix needed.

Component	β-HB Reaction Mix (µL)	β-HB Sample Background Mix (µL)
β-HB Assay Buffer	45	47
β-HB Reagent	3	3
β-HB Enzyme Mix	2	-

2. Add 50 µL of the β-HB Reaction Mix to Sample(s) and β-HB Standard wells and 50 µL of the Sample Background Mix to BC wells. The total reaction volume should be 100 µL/well. Gently tap the plate to mix.
3. Incubate the plate at RT for **15 min**, protected from light.
4. Add 5 µL Developer Solution II into all β-HB wells including Sample, Sample Background Control and β-HB Standard wells.
5. Incubate at RT for 30 min, protected from light.
6. Measure the absorbance (OD) of all wells at 450 nm in endpoint mode.

Acetoacetate Assay:
Standard Preparation

ΔNOTE: Always prepare a fresh set of standards for every use & discard working standard dilutions after use as they do not store well.

1. Prepare a 5 mM Acetoacetate Standard working solution by mixing 10 µl of 100 mM Acetoacetate Standard with 190 µL of Acetoacetate Assay Buffer II.
2. Add 0, 2, 4, 6, 8, and 10 µL of the diluted 5 mM Acetoacetate Standard into a series of wells.
3. Adjust the volume to 50 µL/well with Acetoacetate Assay Buffer II to generate 0, 10, 20, 30, 40 and 50 nmol/well of Acetoacetate Standard.

ΔNOTE: To improve accuracy and reduce CVs, the standard curve may be prepared in duplicate, in a microplate or microcentrifuge tubes using the table below. Each standard mix has enough volume to set up duplicate readings (2 x 50 µL).

Standard #	5 mM Acetoacetate Standard (µL)	Acetoacetate Assay Buffer II (µL)	Final volume standard in well (µL)	Acetoacetate in well (nmol)
1	0	125	50	0
2	5	120	50	10
3	10	115	50	20
4	15	110	50	30
5	20	105	50	40
6	25	100	50	50

Acetoacetate Assay Procedure

Equilibrate all materials and prepared reagents to RT just prior to use and gently agitate. Assay all standards, controls and samples in duplicate.

7. Make enough Acetoacetate Reaction Mix and Sample Background Mix for the number of assays to be performed, 50 µL per reaction as shown below. Remember to account for the Acetoacetate Standard Curve wells when calculating the amount of Reaction Mix needed.

Component	Acetoacetate Reaction Mix (µL)	Acetoacetate Sample Background Mix (µL)
Acetoacetate Assay Buffer II	45	47
Acetoacetate Reagent	3	3
β-HB Enzyme Mix	2	-

8. Add 50 µL of the Acetoacetate Reaction Mix to Sample(s) and Acetoacetate Standard wells and 50 µL of the Sample Background Mix to BC wells. The total reaction volume should be 100 µL/well. Gently tap the plate to mix.
9. Incubate the plate at RT for **5 min**, protected from light.

ΔNOTE: Incubation time should not exceed 5 min, and must be consistent for both the Standard Curve and Sample wells.

10. Add 5 µL Developer Solution II into all Acetoacetate wells including Sample, Sample Background Control and β-HB Standard wells. Gently tap the plate to mix.
11. Incubate at RT for 30 min, protected from light.
12. Measure the absorbance (OD) of all wells at 450 nm in endpoint mode.

Data analysis

β-HB Assay

1. For the β-HB Standard Curve, subtract the 0 nmol/well Standard absorbance reading from the Standard readings ($\Delta OD_{450} = OD_{standard} - OD_{blank}$). Plot the background-corrected Standard values and calculate the slope of the β-HB Standard Curve.
2. For β-HB Samples, subtract the Background Control readings from paired Sample readings ($OD_S - OD_{BC}$) to obtain the corrected Sample readings.
3. Apply the corrected Sample readings to the background-corrected β-HB Standard Curve to get the $\boldsymbol{B}$ nmol of β-HB in the Sample wells.
4. To determine the concentration of β-HB in the Sample(s), use the following equation:

$$[\beta\text{-HB}] = \frac{\boldsymbol{B}}{\boldsymbol{V}} \times \boldsymbol{D} = nmol/\mu L = mM$$

Where: $\boldsymbol{B}$ is the amount of β-HB (nmol) in test sample(s) from Standard Curve
$\boldsymbol{V}$ is the sample volume (µL) added to the well
$\boldsymbol{D}$ is the sample dilution factor (if applicable, *D* = 1 for undiluted samples)

ΔNOTE: Remember to account for any dilution of the sample made during sample preparation, before adding sample to the sample well, when determining D.

Acetoacetate Assay

5. For the Acetoacetate Standard Curve, subtract the 0 nmol/well Standard absorbance reading from all of the Standard readings ($\Delta OD_{450} = OD_{standard} - OD_{blank}$).
6. Construct a plot of Acetoacetate Standard amount (nmol) versus the absolute value of the net absorbance ($|\Delta OD_{450}|$) and calculate the slope of the Acetoacetate Standard Curve.
7. For Acetoacetate Samples, subtract the Background Control readings from paired Sample readings and take the **absolute value**: ($|OD_S - OD_{BC}|$) to obtain the corrected Sample readings.
8. Apply the corrected Sample readings to the background-corrected Acetoacetate Standard Curve to get the $\boldsymbol{B}$ nmol of Acetoacetate in the Sample wells.
9. To determine the concentration of Acetoacetate in the Sample(s), use the following equation:

$$[\text{Acetoacetate}] = \frac{\boldsymbol{B}}{\boldsymbol{V}} \times \boldsymbol{D} = nmol/\mu L = mM$$

Where: $\boldsymbol{B}$ is the amount of Acetoacetate (nmol) in test sample(s) from Standard Curve
$\boldsymbol{V}$ is the sample volume (µL) added to the well
$\boldsymbol{D}$ is the sample dilution factor (if applicable, *D* = 1 for undiluted samples)

ΔNOTE: Remember to account for any dilution of the sample made during sample preparation, before adding sample to the sample well, when determining D.

Total Ketone Body Concentration

To calculate the total ketone body concentration in a sample, add the concentration of β-Hydroxybutyrate and Acetoacetate using the following equation:

$$\textbf{[Total Ketone Body]} = [\beta\text{-HB}] + [\text{Acetoacetate}] = nmol/\mu L = mM$$

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)