Version 1 Last updated 2 May 2018

ab233498
Histone H3 (mono-methyl K9) Quantification Kit (Colorimetric, Circulating)

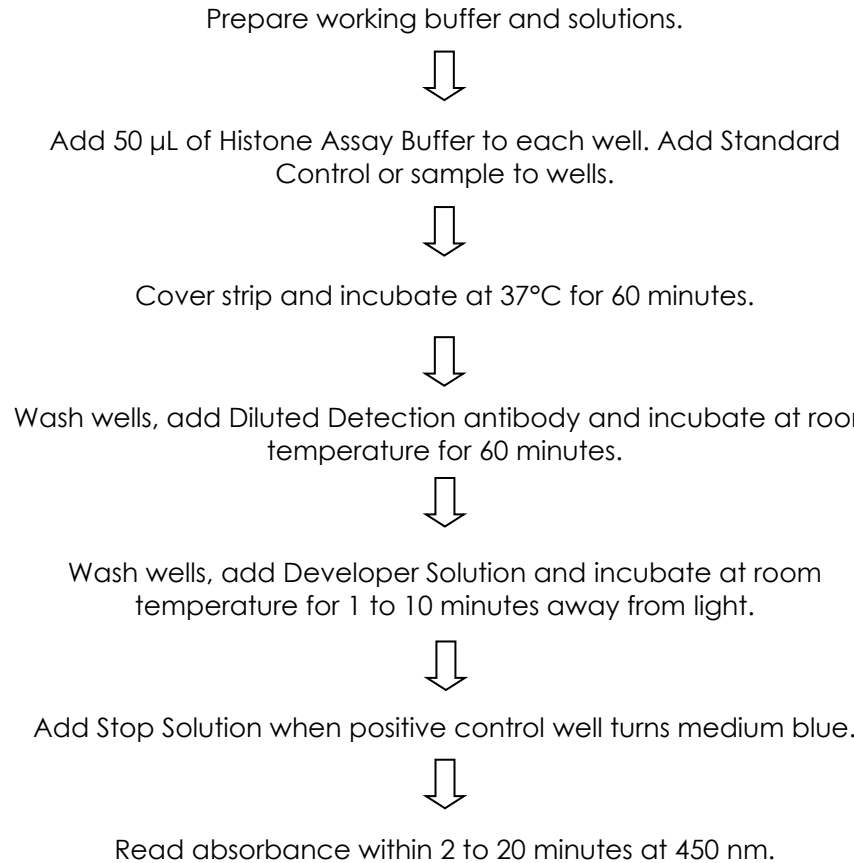
For the measurement of circulating mono-methyl histone H3K9 from biological fluid samples such as plasma and serum from human, mouse or rat.

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

Table of Contents

1. Overview

Histone H3 (mono-methyl K9) Quantification Kit (Colorimetric, Circulating) (ab233498) is designed for measuring total H3K9me1 in plasma or serum. In an assay with this kit, the Histone H3 proteins mono-methylated at K9 in the plasma/serum sample are captured on the strip wells coated with anti-H3K9me1 antibody. The captured H3K9me1 proteins can be then recognized with detection antibody followed by a color development reagent. The ratio of H3K9me1 is proportional to the intensity of absorbance. The absolute amount of H3K9me1 can be quantitated by comparing to the standard control.



2. Materials Supplied and Storage

Store Detection Antibody and Standard Control at –20°C away from light. Store Wash Buffer, Histone Assay Buffer, Developer Solution, 8-Well Assay Strips and Control Assay Strips at 4°C away from light. Store Stop Solution and Adhesive Covering Film at room temperature away from light.

Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity (48 assays)	Quantity (96 assays)	Storage temperature (before prep)
10X Wash Buffer	14 mL	28 mL	4°C
Histone Assay Buffer	4 mL	8 mL	4°C
Developer Solution	5 mL	10 mL	4°C
Stop Solution	5 mL	10 mL	RT
8-Well Assay Strips (With Frame)	4	10	4°C
Control Assay Strips (With Frame)#	2	2	4°C
Adhesive Covering Film	1	1	RT
Detection Antibody, 1000X*	6 µL	12 µL	-20°C
Standard Control (100 µg/mL)	10 µL	20 µL	-20°C

*Spin the solution down to the bottom prior to use.
#Control Assay Strips are green trimmed for distinguishing from 8-well Assay Strips (for samples). The Control Assay Strips are only for control use and should not be used for sample assay.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of reading absorbance at 450 nm.
- Incubator for 37°C incubation.
- Distilled water.
- Plasma or serum.
- Parafilm M or aluminum foil.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

If Wash Buffer contains salt precipitates, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

5.1 10X Wash Buffer

5.1.1 For a 48-reaction size kit, prepare diluted 1X Wash Buffer by adding 13 mL of 10X Wash Buffer to 117 mL of distilled water and adjust pH to 7.2-7.5.

5.1.2 For the 96-reaction size kit, add 26 mL of 10X Wash Buffer to 234 mL of distilled water and adjust pH to 7.2-7.5.
Δ Note: Diluted 1X Wash Buffer can now be stored at 4°C for up to six months.

5.2 Detection Antibody

Prepare diluted Detection Antibody with 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µL of Detection Antibody to 1000 µL of 1X Wash Buffer). 50 µL of diluted Detection Antibody will be required for each assay well.
Δ Note: Keep the diluted solution on ice until use. Any remaining diluted solution should be discarded if not used within the same day.

5.3 Histone Assay Buffer

Ready to use as supplied.

5.4 Developer Solution

Ready to use as supplied.
Δ Note: Check if a blue color is present in Developer Solution, which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of Developer Solution required into a secondary container (tube or vial) before adding Developer Solution into the assay wells.

5.5 Stop Solution

Ready to use as supplied.

5.6 8-Well Assay Strips (With Frame)

Ready to use as supplied.

5.7 Control Assay Strips (With Frame)

Ready to use as supplied. Control Assay Strips are green trimmed for distinguishing from 8-well Assay Strips (for samples). The Control Assay Strips are only for control use and should not be used for sample assay.

5.8 Standard Control

Ready to use as supplied. Spin the solution down to the bottom prior to use.

6. 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. First, dilute Standard Control to 50 ng/µL by adding 5 µL of Standard Control to 5 µL of Histone Assay Buffer and to 5 ng/µL by adding 1 µL of Standard Control to 19 µL of Histone Assay Buffer.
2. Then, further prepare seven concentrations by using the 5 ng/µL and 50 ng/µL of Diluted Standard Control with Histone Assay Buffer into final concentrations of 0.5, 1, 2, 5, 10, 20, and 50 ng according to the following dilution chart:

Tube	Standard Control (5 ng/µL)	Standard Control (50 ng/µL)	Histone Assay Buffer	Resulting Concentration
1	1 µL		9 µL	0.5 ng/µL
2	1 µL		4 µL	1 ng/µL
3	2 µL		3 µL	2 ng/µL
4	4 µL		0 µL	5 ng/µL
5		1 µL	4 µL	10 ng/µL
6		2 µL	3 µL	20 ng/µL
7		4 µL	0 µL	50 ng/µL

Δ Note: Keep each of the diluted solutions on ice until use. Any remaining diluted solutions should be discarded if not used within the same day.

7. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay.

Input materials should be plasma or serum. The amount of plasma or serum for each assay can be 10 to 40 µL with an optimal amount of 30 µL.

The standard control is provided in this kit for the quantification of circulating histone H3K9me1. Because content of H3K9me1 can vary from different individuals and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated.

8. Assay Procedure

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

8.1 Histone Binding

1. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove unneeded strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).
2. Blank Wells: Add 50 µL of Histone Assay Buffer to each blank well.
3. Standard Wells: Add 50 µL of Histone Assay Buffer and 1 µL of Diluted Standard Control to each standard well, each at a different concentration between 0.5 and 50 ng/µL (based on the dilution chart in Step 6.1 Standard Preparation.
4. Sample Wells: Add 50 µL of Histone Assay Buffer and 30 µL of your plasma or serum sample.
   Δ Note: Follow the suggested well setup diagrams as indicated in Table 1.
5. Tightly cover strip-well microplate with Adhesive Covering Film to avoid evaporation and incubate at 37°C for 60 minutes.
   Δ Note: The Adhesive Covering Film can be cut to the required size to cover the strips based on the number of strips to be used.
6. Remove the reaction solution from each well. Wash each well three times with 150 µL of 1X Wash Buffer each time.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	SC 0.5 ng	SC 0.5 ng	Sample	Sample	Sample	Sample
C	SC 1 ng	SC 1 ng	Sample	Sample	Sample	Sample
D	SC 2 ng	SC 2 ng	Sample	Sample	Sample	Sample
E	SC 5 ng	SC 5 ng	Sample	Sample	Sample	Sample
F	SC 10 ng	SC 10 ng	Sample	Sample	Sample	Sample
G	SC 20 ng	SC 20 ng	Sample	Sample	Sample	Sample
H	SC 50 ng	SC 50 ng	Sample	Sample	Sample	Sample

Table1. The suggested strip-well plate setup for H3K9me1 quantification in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate. Strip 1 and Strip 2 are the green trimmed control strips.

8.2 Detection Antibody Binding

1. Prepare Diluted Detection Antibody as indicated above in 'Reagent Preparation 5.2'. Add 50 µL of the Diluted Detection Antibody to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 minutes.
2. Remove the Diluted Detection Antibody solution from each well.
3. Wash each well four times with 150 µL of the 1X Wash Buffer each time.
   Δ Note: Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.

8.3 Signal Detection:

1. Add 100 µL of Developer Solution to each well and incubate at room temperature for 1 to 10 minutes away from light. Begin monitoring color change in the sample wells and control wells. The Developer Solution will turn blue in the presence of sufficient H3K9me1 product.
2. Add 100 µL of Stop Solution to each well to stop enzyme reaction when the color in the positive control wells turns medium blue. The color will change to yellow after adding Stop Solution and the absorbance should be read on a microplate reader within 2 to 10 minutes at 450 nm with an optional reference wavelength of 655 nm.

Δ Note: Most microplate readers have capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice – once at 450 nm and once at 655 nm. Then manually subtract the 655 nm ODs from 450 nm ODs.

Δ Note: If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

9. Data Analysis

Calculate the average for duplicate readings for sample wells and blank wells.

Calculate % H3K9me1 change using the following formula if the samples are from treated and un-treated control tests.

For simple calculation:

$$H3K9me1\% = \frac{Treated\ (Tested) Sample\ OD - Blank\ OD}{Untreated\ (Control) Sample\ OD - Blank\ OD} \times 100\%$$

Example calculation:

Average OD450 of treated sample is 0.3

Average OD450 of untreated control is 0.4

Average OD450 of blank is 0.1

$$H3K9me1\% = \frac{(0.3 - 0.1)}{(0.4 - 0.1)} \times 100\ \% = 66.7\ \%$$

For accurate calculation:

1. Generate a standard curve and plot OD value versus amount of Standard Control at each concentration point.
2. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of H3K9me1 using the following formulas:

$$H3K9me1\ (ng/ml) = \frac{(Sample\ OD - Blank\ OD)}{Slope \text{ x Sample amount (ul *)}} \times 100\%$$

* Plasma or serum added into sample wells at Step 8.1.4.

10. Typical Data

Data provided **for demonstration purposes only.**

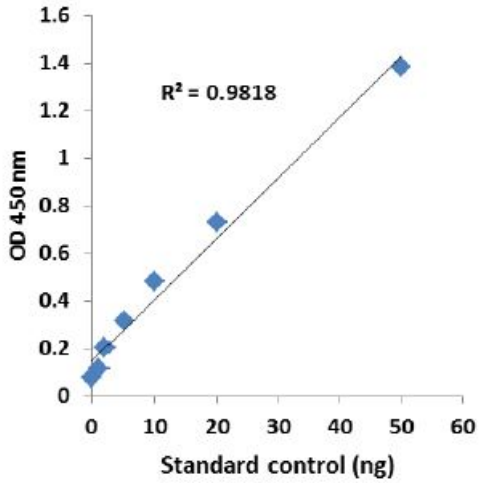


Figure 1. Example standard curve.

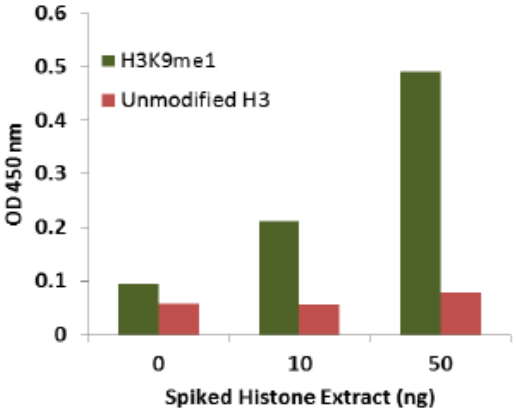


Figure 2. Histone extracts were prepared from HL-60 cells and spiked into bovine plasma at different concentrations. The amount of H3K9me1 was measured using the ab233498 Histone H3 (mono-methyl K9) Quantification Kit (Colorimetric, Circulating).

11. FAQs / Troubleshooting

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly secure after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 8.1.3.	Ensure a sufficient amount of standard is added.
	The standard is degraded due to improper storage conditions.	Follow the Materials supplied and Storage guidance for storage of Standard Control.
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step are performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.
	Incubation time with Detection Antibody is too long.	The incubation time at Step 8.2.1 should not exceed 90 minutes.

High background present in the blank wells	Over development of color.	Decrease the development time in Step 8.3.1 before adding Stop Solution in Step 8.3.2
No signal or weak signal only in sample wells.	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of plasma or serum is used as indicated in Step 8.1.4.
	Sample was not stored properly or has been stored for too long.	Ensure plasma or serum is stored in aliquots at proper temperature, for no more than 6 months.
	Little or no H3K9me1 in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared samples.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and that residue washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution or stop solutions is added sequentially and consistent with the order you added the other reagents.

12. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829