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ab233496
Histone H3 (di-methyl K4) Quantification Kit (Colorimetric, Circulating)

For detecting circulating dimethyl histone H3K4 (H3K4me2) 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.

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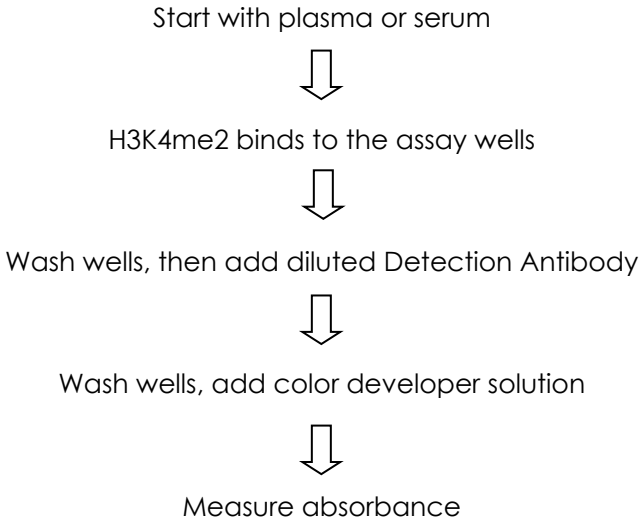
1. Overview

Histone H3 (di-methyl K4) Quantification Kit (Colorimetric, Circulating) (ab233496) is a convenient package of tools designed to specifically measure circulating dimethyl histone H3K4 (H3K4me2) from biological fluid samples such as plasma and serum from human, mouse or rat. This kit only recognizes H3K4me2 with no cross-reactivity with unmodified H3 or other modifications at the same lysine site. The amount of plasma or serum for each assay can be 10-40 µl with an optimal amount of 30 µl.



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, Control Assay Strips and 8-Well 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 6 months 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	48 tests Quantity	96 tests Quantity	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	4	10	4°C
Control Assay Strips	2	2	4°C
Adhesive Covering Film	1	1	RT
Detection Antibody	6 µL	12 µL	-20°C
Standard Control	10 µL	20 µL	-20°C

Δ Note: *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
- Aluminum foil or Parafilm M
- Distilled water
- Sample of interest (plasma or serum)

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.
- Check if a blue color is present in the 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 it into the assay wells.

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 Histone Assay Buffer

Ready to use as supplied.

5.3 Developer Solution

Ready to use as supplied.

5.4 Stop Solution

Ready to use as supplied.

5.5 8-Well Assay Strips

Ready to use as supplied.

5.6 Control Assay Strips

Ready to use as supplied.

5.7 Adhesive Covering Film

Ready to use as supplied.

5.8 Detection Antibody

Dilute 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). About 50 µL of this diluted Detection Antibody will be required for each assay well).

5.9 Standard Control

Prepare just before assay (Step 6).

Δ Note *Keep each of the individual solutions (except diluted 1X Wash Buffer) on ice until use. Any remaining diluted solutions, other than the diluted 1X Wash Buffer, should be discarded if not used within the same day.*

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. 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. 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	1.0 µL	---	9.0 µL	0.5 ng/µL
2	1.0 µL	---	4.0 µL	1 ng/µL
3	2.0 µL	---	3.0 µL	2 ng/µL
4	4.0 µL	---	0 µL	5 ng/µL
5	---	1.0 µL	4.0 µL	10 ng/µL
6	---	2.0 µL	3.0 µL	20 ng/µL
7	---	4.0 µL	0.0 µL	50 ng/µL

Δ Note *Keep each of the diluted solutions (except 1X Wash Buffer) on ice until use. Any remaining diluted solutions, other than 1X Wash Buffer, should be discarded if not used within the same day.*

7. 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.

7.1 Histone Binding:

1. Predetermine the number of strip-wells required for your experiment. Carefully remove un-needed 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 below).
4. Sample Wells: Add 50 µl of Histone Assay Buffer and 30 µl of your plasma or serum sample.

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

Δ Note: *The suggested strip-well plate setup for H3K4me2 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.*

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 the 1X Wash Buffer each time.

7.2 Detection Antibody Binding:

1. Add 50 µl of the diluted Detection Antibody (from Step 5.8) to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 minutes.
   Δ Note: *Do not exceed incubation time of 90 minutes.*
2. Remove the diluted Detection Antibody 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.*

7.3 Signal Detection:

1. Add 100 µl of Developer Solution to each well and incubate at room temperature for 1-10 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 H3K4me2 product.
2. Add 100 µl of Stop Solution to each well to stop enzyme reaction when 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 2-10 minutes at 450 nm with an optional reference wavelength of 655 nm.
   Δ Note: *Most microplate readers have the 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.*

8. Data Analysis

H3K4me2 Calculation: Calculate the average duplicate readings for the sample wells and blank wells. Calculate % H3K4me2 change using the following formula if the samples are from treated and un-treated control tests:

$$\text{H3K4me2\%} = \frac{\text{Treated (Tested) Sample OD - Blank OD}}{\text{Untreated (Control) Sample OD - Blank OD}} * 100\%$$

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

$$\text{H3K4me2\%} = \frac{0.3 - 0.1}{0.4 - 0.1} * 100\% = 66.7\%$$

Accurate Calculation: Generate a standard curve and plot OD value versus amount of Standard Control at each concentration point. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of H3K4me2 using the following formula:

$$\text{H3K4me2(ng/ml)} = \frac{\text{(Sample OD - Blank OD)}}{\text{Slope x sample amount (µl *)}} \text{ x } 1000$$

* Plasma or serum added into the sample wells at Step 7.1.4.

Δ Note: *To measure the content of H3K4me2 in total histone H3 for normalizing accuracy of the quantified H3K4me2 %, total histone H3 amount in the samples should be quantified.*

9. Typical Data

Data provided **for demonstration purposes only**.

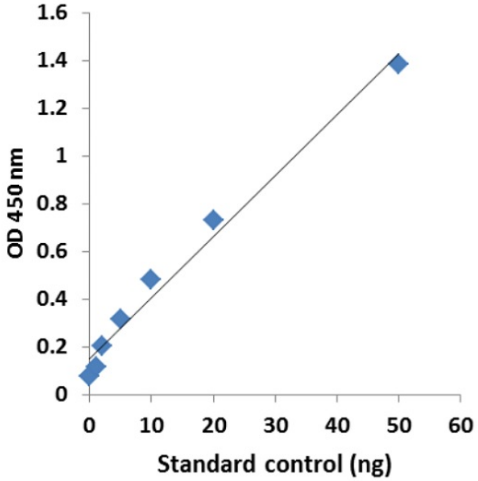


Figure 1. An example of an optimal standard curve generated with Histone H3 (di-methyl K4) Quantification Kit (Colorimetric, Circulating) (ab233496).

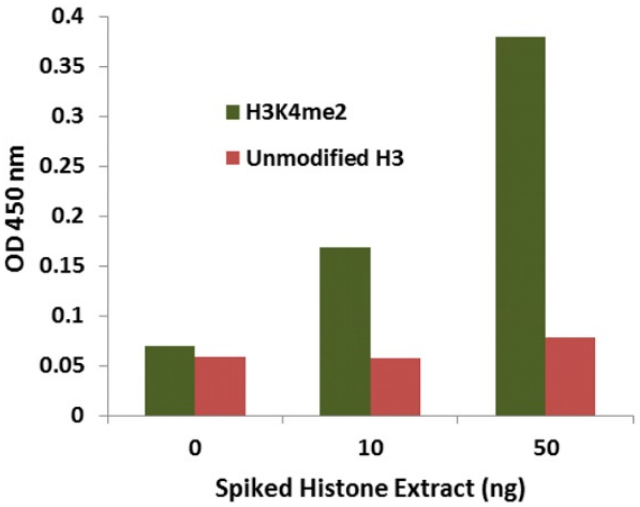


Figure 2. Histone extracts were prepared from HL-60 (Human promyelocytic leukemia cell line) cells and spiked into bovine plasma at different concentrations. The amount of H3K4me2 was measured using Histone H3 (di-methyl K4) Quantification Kit (Colorimetric, Circulating) (ab233496).

10. 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 temperatures and the caps are tightly fastened 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 7.1.3.	Ensure a sufficient amount of standard is added.
	The standard is degraded due to	Follow the Shipping & Storage guidance in

	improper storage conditions.	this User Guide 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 7.2.1 should not exceed 90 minutes.
	Over-development of color.	Decrease the development time in Step 7.3.1 before adding Stop Solution in Step 7.3.2.
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 7.1.4.
	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 H3K4me2 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 solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12).

11. Notes

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