

Version 1 Last updated 9 April 2018

ab233488 m6A DNA Methylation Assay Kit (Colorimetric)

For the measurement of m6A DNA methylation in mammals, plants, fungi, bacteria, and viruses.

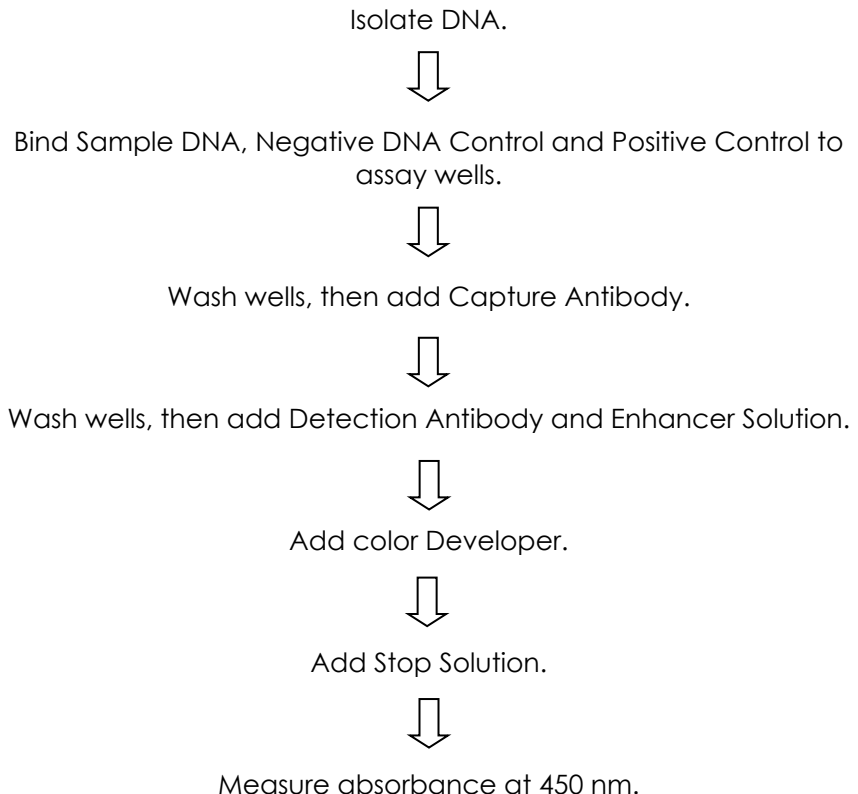
This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview	3
2. Materials Supplied and Storage	4
3. Materials Required, Not Supplied	5
4. General guidelines, precautions, and troubleshooting	6
5. Reagent Preparation	7
6. Standard Preparation	9
7. Sample Preparation	10
8. Assay Procedure	11
9. Data Analysis	14
10. Typical Data	16
11. Troubleshooting	18
12. Notes	20

1. Overview

m6A DNA Methylation Assay Kit (Colorimetric) (ab233488) contains all reagents necessary for the quantification of m6A in DNA. In this assay, DNA is bound to strip wells using DNA high binding solution. m6A is detected using capture and detection antibodies. The detected signal is enhanced and then quantified colorimetrically by reading the absorbance in a microplate spectrophotometer. The amount of m6A is proportional to the OD intensity measured.



2. Materials Supplied and Storage

Store Negative Control, Positive Control, Detection antibody and Enhancer Solution at -20°C protected from light. Store 10X Wash Buffer, Capture Antibody, Developer Solution and 8-Well Assay Strips at 4°C protected 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	48 tests Quantity	96 tests Quantity	Storage temperatur e (before prep)
10X Wash Buffer	14 mL	28 mL	4°C
Binding Solution	5 mL	10 mL	RT
Negative DNA Control, 100 $\mu\text{g}/\text{mL}$	10 μL	20 μL	-20°C
Positive Control containing 1 $\mu\text{g}/\text{mL}$ m ⁶ A, 200 $\mu\text{g}/\text{mL}$	10 μL	20 μL	-20°C
Capture Antibody, 1000X	5 μL	10 μL	4°C
Detection Antibody, 1000X	6 μL	12 μL	-20°C
Enhancer Solution, 1000X	5 μL	10 μL	-20°C
Developer Solution	5 mL	10 mL	4°C
Stop Solution	5 mL	10 mL	RT
8-Well Assay Strips (With Frame)	6	12	4°C

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.
- 1.5 mL microcentrifuge tubes.
- Plate seal or Parafilm M.
- Distilled water.
- 1X TE buffer pH 7.5 to 8.0.
- Isolated DNA of interest.

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.

5.1 10X Wash Buffer

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.
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 be stored at 4°C for up to six months.

5.2 Binding Solution

Ready to use as supplied.

5.3 1000X Capture Antibody

Dilute 1000X Capture Antibody 1:1000 with 1X Wash Buffer (1 μ L 1000X Capture antibody to 1000 μ L 1X Wash Buffer).

ΔNote: Each assay well requires approximately 50 μ L of diluted Capture Antibody.

5.4 1000X Detection Antibody

Dilute 1000X Detection Antibody 1:2000 with 1X Wash Buffer (1 μ L 1000X Capture Antibody to 2000 μ L 1X Wash Buffer).

ΔNote: Each assay well requires approximately 50 μ L of diluted Detection Antibody.

5.5 Enhancer Solution

Dilute Enhancer solution 1:5000 (1 μ L 1000X Enhancer Solution to 5000 μ L 1X Wash Buffer).

5.6 Developer Solution

Ready to use as supplied.

5.7 Positive Control containing 1 µg/mL m⁶A, 200 µg/mL
See Standard Preparation (Section 6).

5.8 Stop Solution
Ready to use as supplied.

5.9 8-Well Assay Strips (With Frame)
Ready to use as supplied.

Suggested working buffer and solution set up

Reagent	1 well	8 wells (1 strip)	16 wells (2 strips)	48 wells (6 strips)	96 wells (12 strips)
Diluted Wash Buffer	2.5 mL	20 mL	40 mL	120 mL	240 mL
Binding Solution	80 µL	640 µL	1.3 mL	3.9 mL	8 ml
Diluted Capture Antibody	50 µL	400 µL	800 µL	2.4 mL	4.8 mL
Diluted Detection Antibody	50 µL	400 µL	800 µL	2.4 mL	4.8 mL
Diluted Enhancer Solution	50 µL	400 µL	800 µL	2.4 mL	4.8 mL
Detection Solution	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL
Stop Solution	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL
Negative DNA Control	N/A	0.5-1 µL	0.5-2 µL	1-4 µL	2-8 µL
Positive	N/A	0.5-1 µL	0.5-2 µL	1-4 µL	2-8 µL

Control					
---------	--	--	--	--	--

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. **Single point control:** Dilute the Positive Control with 1X TE buffer (not supplied) to 0.5 ng/μL (e.g. 2 μL Positive Control + 2 μL 1X TE buffer).
 2. **Standard Curve (recommended):** First dilute Positive Control to 0.5 ng/μL (e.g. 5 μL Positive Control + 5 μL 1X TE buffer) and 0.1 ng/mL (e.g. 1 μL Positive Control + 9 μL 1X TE buffer).
Next, prepare 7 different concentrations as detailed in the table.

Tube	Positive Control (0.5 ng/μL)	1X TE Buffer	Positive Control (0.1 ng/μL)	Final Positive Control Concentration (ng/μL)
1	0 μL	19.5 μL	0.5 μL	0.002
2	0 μL	9.5 μL	0.5 μL	0.005
3	0 μL	9 μL	1.0 μL	0.01
4	0 μL	4 μL	1.0 μL	0.02
5	0 μL	3 μL	3.0 μL	0.05
6	1.0 μL	4 μL	0 μL	0.1
7	2.0 μL	3 μL	0 μL	0.2

ΔNote: Keep each of the diluted solutions (except diluted Wash Buffer) on ice until use. Any remaining standard solutions should be discarded if not used on the same day as they are prepared.

7. Sample Preparation

General sample information:

Input DNA quality and amount:

Input DNA should be highly pure with 260/280 ratio >1.8 and relatively free of RNA. RNAase A can be used to remove RNA. Starting DNA may be in water or TE buffer.

The DNA amount can range from 100 ng to 300 ng per reaction, with 200 ng being optimal.

ΔNote: The abundance of m6A is generally less than 0.05% of total DNA.

DNA Isolation:

You can use your method of choice for DNA isolation. Abcam offers a series of genomic DNA isolation kits for your convenience.

DNA Storage:

Isolated genomic DNA can be stored at -20°C (short term) or -80°C (long term) until use.

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.
- Review the configuration of the strip-well plate setup for standard curve preparation in a 48-assays format below (for a 96-reaction format, strips 7 through 12 can be configured as Sample). The controls and samples can be measured in duplicate, loaded vertically instead of horizontally.

Single point Positive Control

Well	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	NC	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19
B	NC	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19
C	PC	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20
D	PC	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20
E	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21
F	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21
G	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22
H	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22

Standard Curve Positive Control

Well	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	NC	PC 0.04 ng/ well	Sample 1	Sample 5	Sample 9	Sample 13
B	NC	PC 0.04 ng/ well	Sample 1	Sample 5	Sample 9	Sample 13
C	PC 0.004 ng/ well	PC 0.1 ng/ well	Sample 2	Sample 6	Sample 10	Sample 14
D	PC 0.004 ng/ well	PC 0.1 ng/ well	Sample 2	Sample 6	Sample 10	Sample 14
E	PC 0.01 ng/ well	PC 0.2 ng/ well	Sample 3	Sample 7	Sample 11	Sample 15
F	PC 0.01 ng/ well	PC 0.2 ng/ well	Sample 3	Sample 7	Sample 11	Sample 15
G	PC 0.02 ng/ well	PC 0.4 ng/ well	Sample 4	Sample 8	Sample 12	Sample 16
H	PC 0.02 ng/ well	PC 0.4 ng/ well	Sample 4	Sample 8	Sample 12	Sample 16

8.1 DNA 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. Add 80 µL of Binding Solution to each well.
3. Add 2 µL Negative DNA Control, 2 µL Positive Control, and 200 ng of Sample DNA (1-8 µL) into the appropriate wells (see Tables, above). Mix solution by gently tilting from side-to-side or shaking the plate several times. Ensure the solution coats the bottom of the plate evenly.
4. Cover strip plate with plate seal or Parafilm M and incubate at 37°C for 90 minutes.
5. Remove the Binding Solution from each well. Wash each well with 150 µl of Diluted Wash Buffer by pipetting Diluted Wash Buffer into the wells and then removing it using a pipette. Repeat the wash two times for a total of three washes.

8.2 m6A DNA Capture:

1. Add 50 µl of Diluted Capture Antibody to each well, then cover and incubate at room temperature for 60 minutes.
2. Remove the Diluted Capture Antibody solution from each well using a pipette.
3. Wash each well with 150 µl of Diluted Wash Buffer each time for three times.
4. Add 50 µl of Diluted Detection Antibody to each well, then cover and incubate at room temperature for 30 minutes.
5. Remove the Diluted Detection Antibody solution from each well using a pipette.
6. Wash each well with 150 µl of Diluted Wash Buffer each time for four times.
7. Add 50 µl of Diluted Enhancer Solution to each well, then cover and incubate at room temperature for 30 minutes.
8. Remove the Diluted Enhancer Solution solution from each well.
9. Wash each well with 150 µl of Diluted Wash Buffer each time for five times.

8.3 Signal Detection:

1. Add 100 µl of Detection Solution to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The Detection Solution solution will turn blue in the presence of sufficient m6A.
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 at 450 nm within 2 to 15 min.

9. Data Analysis

Relative Quantitation:

To determine the relative m6A DNA methylation status of two different DNA samples, a simple calculation for the percentage of m6A in your DNA can be carried out using the following formula:

$$\text{m6A \%} = \frac{(\text{Sample OD} - \text{NDC OD})/S}{(\text{Positive Control OD} - \text{NDC OD})/P} * 100\%$$

Where:

S = amount of input Sample DNA in ng

P = amount of input Positive Control in ng

NDC = Negative DNA Control

OD = Optical density

Example Calculation:

Average OD₄₅₀ of NDC is 0.1

Average OD₄₅₀ of Positive Control is 0.6

Average OD₄₅₀ of Sample id 0.14

Sample is 200 ng

Positive Control is 1 ng

$$\text{m6A\%} = \frac{(0.14 - 0.1)/200}{(0.6 - 0.1)/1} \times 100\% = 0.04\%$$

Absolute Quantitation:

To quantify the absolute amount of m6A using an accurate calculation, first generate a standard curve and plot the OD values (background (NC)-subtracted) versus the amount of PC at each concentration point. Next, determine the slope (OD/ng) of the standard curve using linear regression (Microsoft Excel's linear

regression functions are suitable for such calculation). Use the most linear part of the standard curve (include at least 4 concentration points) for optimal slope calculation. Now calculate the amount and percentage of m6A in your DNA using the following formulas:

$$m6A \text{ (ng)} = \frac{\text{Sample OD} - \text{Negative Control OD}}{\text{Slope}}$$

$$m6A \% = \frac{m6A \text{ amount (ng)}}{S} * 100\%$$

S is the amount of input Sample DNA in ng.

Example calculation:

Average OD450 of NC is 0.10

Average OD450 of sample is 0.14

Slope is 0.5 OD/ng

S is 200 ng

$$m6A \text{ (ng)} = \frac{0.14 - 0.1}{0.5} = 0.08ng$$

$$m6A \% = \frac{0.08}{200} \times 100\% = 0.04\%$$

10. Typical Data

Data provided for demonstration purposes only.

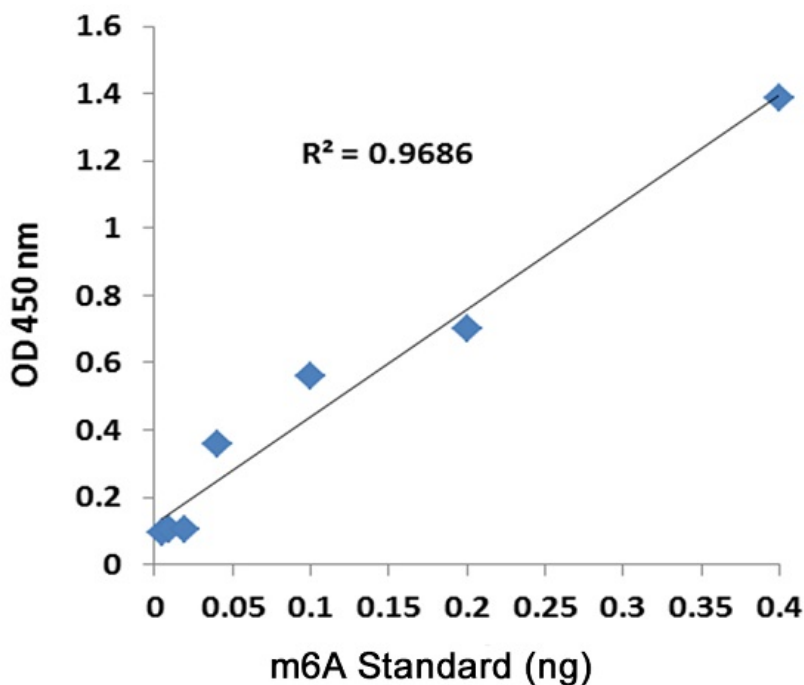


Figure 1. m6A standard control was added into the assay wells at different concentrations and then measured with the m6A DNA Methylation ELISA Kit (Colorimetric) (ab233488).

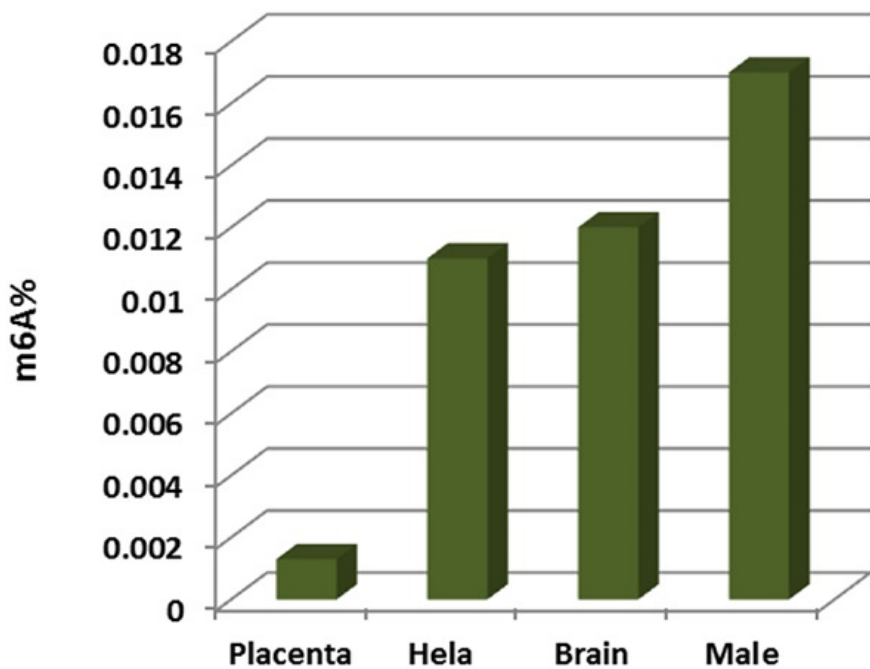


Figure 2. Quantification of m 6A content of various human DNA samples with the m6A DNA Methylation ELISA Kit (Colorimetric) (ab233488).

11. Troubleshooting

Problem	Reason	Solution
No signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order and if any steps in the protocol may have been omitted by mistake.
	The well is incorrectly washed before DNA binding.	Ensure the well is not washed prior to adding the positive control and sample.
	The bottom of the well is not completely covered by the Binding Solution.	Ensure the solution coats the bottom of the well by gently tilting from side to side or gently shaking the plate several times.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Insufficient input materials.	Ensure that a sufficient amount of positive control (> 0.2 ng) and sample (200 ng) is added into the wells.
	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 caps are tightly capped after each opening or use.
No signal or weak signal in only the positive control wells	The Positive Control is insufficiently added to the well.	Ensure a sufficient amount of Positive Control is added.
	The Positive Control is degraded due to improper storage conditions.	Follow the Shipping & Storage guidance in this User Guide for storage of Positive Control.

High background present in the negative control wells	Insufficient washing of wells.	Check if washing recommendations at each step are performed according to the protocol.
	Contaminated by sample or Positive Control.	Ensure the well is not contaminated from adding sample or Positive Control accidentally or from using contaminated tips.
	Incubation time is too long.	The incubation time at Step 8.1.4 should not exceed 2 hours.
	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	DNA sample is not properly extracted or purified.	Ensure the DNA sample is good quality. The 260/280 ratio should be >1.8 with no or minimal RNA contamination.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of DNA is used.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the washing guidelines. Make sure the residue of the 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).

12. Notes

Technical Support

Copyright © 2012-2017 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

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