ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quest 5-hmC™ DNA ELISA Kit

Catalog Nos. **D5425 & D5426**

Highlights

- Sensitive and specific quantitation of 5-hydroxymethylcytosine (5-hmC) DNA from a variety of samples.
- Ideal for global 5-hmC detection, tissue-specific 5-hmC quantitation, high-throughput compound screening, and more.
- Streamlined workflow can be completed in as little as 3 hours.

Contents

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

Product Contents

Quest 5-hmC™ DNA ELISA Kit	D5425 (1 × 96 wells)	D5426 (2 × 96 wells)	Storage Temperature
Coating Buffer	15 ml	30 ml	4 °C
10X ELISA Buffer	30 ml	60 ml	4 °C
Anti-5-Hydroxymethylcytosine Polyclonal Antibody (1 mg/ml)	25 µl	50 µl	-20 °C
Anti-DNA HRP Antibody (100X)	100 µl	200 µl	-80 °C
HRP Developer	15 ml	30 ml	4 °C
Control DNA Set (5 Controls)	5 × 40 µl	5 × 40 µl	-20 °C
96-well ELISA Plate (12 x 8-well Strips)	1 plate	2 plates	Room Temp.
Protocol	1	1	-

Note- Integrity of kit components is guaranteed for up to six (6) months from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

Specifications

- **Sample Sources** – This ELISA procedure has been optimized for the detection of 5-hmC in purified genomic DNA that is intact, sheared or fragmented in PBS, Tris-EDTA, or water. The product is also compatible with DNA from other sources.

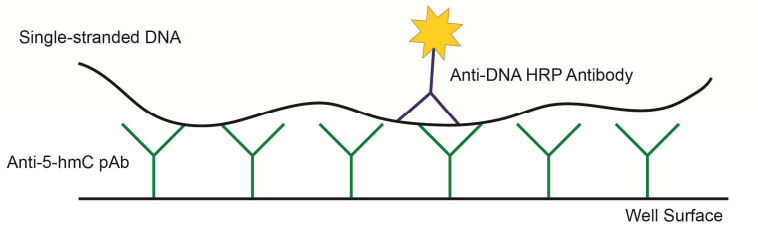
- **Detection** – This system is highly sensitive for 5-hmC DNA and has a lower detection of 0.02% per 100 ng input DNA, making it easy to detect even the smallest percentage of 5-hmC in DNA samples**.**

- **Equipment Required** – An incubator and plate reader (w/ 405-450 nm detection) are required. A multi-channel pipettor is recommended. An automated plate washer may be used for blocking and wash steps due to the one-buffer system.

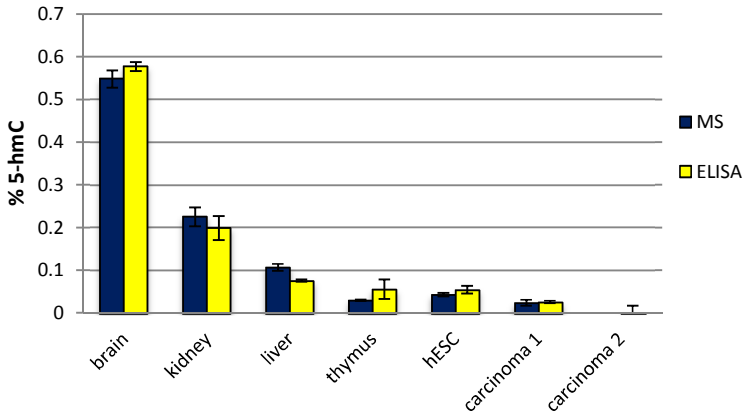
Product Description

The 5-methylcytosine (5-mC) modification of DNA in epigenetic regulation has been well studied over the last several decades. However, the role of the so-called “sixth base”, 5-hydroxymethylcytosine (5-hmC), has yet to be defined. Recent studies have associated 5-hmC patterns to transcriptional regulation of genes, which may contribute to normal and disease states of organisms.

The **Quest 5-hmC™ DNA ELISA Kit** is both sensitive and specific and can be used to accurately detect 5-hmC DNA in a variety of samples. The kit is compatible with a wide range of input DNA including intact vertebrate, plant, and microbial genomic DNA, as well as enzyme-digested and mechanically sheared fragments. The **Control DNA Set** included with this kit has been calibrated to accurately quantify the percent 5-hmC in sample DNA by use of a standard curve. Also, the fast, streamlined workflow is ideal when analyzing/screening large numbers of samples.



Quest 5-hmC™ DNA ELISA Kit is a sandwich-based ELISA format. First, **Anti-5-Hydroxymethylcytosine Polyclonal Antibody** (anti-5-hmC pAb) is coated to the bottom of a well. Single stranded 5-hmC-containing DNA binds to anti-5-hmC pAb which is then recognized by **Anti-DNA HRP Antibody**. Addition of **HRP Developer** will produce a greenish-blue color in the wells containing 5-hmC DNA.



The Quest 5-hmC™ DNA ELISA Kit can be used to detect 5-hmC in numerous DNA samples with high specificity as evidenced by comparison with LC-MS/MS-MRM analysis. 5-hmC pAb (200 ng/well) was used to quantitate the amount 5-hmC in 100 ng of single-stranded DNA. For this, % 5-hmC was calculated from a standard curve generated using the **Control DNA Set**. The figure shows a correlation between the % 5-hmC in DNA samples calculated using the **Quest 5-hmC™ DNA ELISA Kit** (ELISA) and mass spectrometry (MS).

For **Technical Assistance**, please contact Zymo Research Technical Support at: 1-888-882-9682 or e-mail tech@zymoresearch.com.

Experimental Considerations

All DNA used with the kit must be denatured prior to use. The protocol is optimized for the detection of 5-hmC in 100 ng of single-stranded DNA/well. Depending on your experimental design, the amount of input DNA can range from 25-200 ng/well without influencing the detection and quantification of 5-hmC.

The Control DNA Set consists of five double stranded genomic DNA controls containing a specified percentage of 5-hmC. Each control is provided at a concentration of 100 ng/µl. For 5-hmC detection, not all controls have to be used. For example: **Control A** (0%) can serve as a negative control, and **Control E** (0.55%) as a positive control. However, for accurate quantification of 5-hmC percentage, a standard curve must be generated using all controls (see Appendix A, page 5).

Buffer Preparation and Storage

- ✓ Prepare the **1X ELISA Buffer**, pH 7.4, by diluting the **10X ELISA Buffer** solution (1:10) in deionized water. The **1X ELISA Buffer** may be prepared all at once and stored at 4°C for use within one week, or aliquotted and stored at -20°C for up to six months. Repeated freeze/thaw cycles should be avoided.
- ✓ The **Coating Buffer**, pH 9.6, is ready for use and is stable at room temperature or 4 °C for extended periods of time.
- ✓ The **HRP Developer** is also ready for use and should be stored at 4°C. For more rapid color development, bring **HRP Developer** to room temperature before adding to the wells of the ELISA plate.
- ✓ **Anti-DNA HRP Antibody** can be stored at -20°C for 1 week. For long term storage, the antibody should be kept at -80°C. Avoid freeze/thaw cycles.

Protocol*

*It is recommended that samples and controls be assayed in **duplicate** for accurate 5-hmC detection in DNA.*

Coating:

1. Remove the amount of 8-well strips required to assay samples and standards[1].
2. Dilute **Anti-5-Hydroxymethylcytosine Polyclonal Antibody** (1 mg/ml) to 1 ng/µl in **Coating Buffer**.
3. Add 100 µl/well of the diluted anti-5-hmC pAb[2]. Cover the plate with foil to prevent evaporation and incubate at 37 °C for 1 hour.

Blocking:

1. Discard buffer from the wells of the plate. Wash each well with 200 µl of **1X ELISA Buffer** and remove liquid from each well by tapping out excess liquid onto a paper towel. Repeat this wash step 2 more times.
2. Add 200 µl of **1X ELISA Buffer** to each well. Cover the plate with foil and incubate at 37 °C for 30 minutes.

DNA Addition/Binding:

1. Denature the sample and control DNAs at 98°C for 5 minutes[3] in a thermocycler.
2. Immediately transfer samples to ice for 10 minutes[4].
3. Dilute single-stranded samples and control DNAs to a final concentration 1 ng/µl in **1X ELISA Buffer**.
4. Discard buffer from the wells of the plate. Remove all liquid from each well by tapping out excess liquid onto a paper towel.
5. Add 100 µl of the diluted sample and standard DNAs to each well[5]. Cover the plate with foil and incubate at 37 °C for 1 hour.

Addition Anti-DNA HRP Antibody:

1. Discard buffer from the wells of the plate. Wash each well with 200 µl of **1X ELISA Buffer**. Remove all liquid from each well by tapping out excess liquid onto a paper towel. Repeat this wash step 2 more times.
2. Prepare a 1:100 final dilution of **Anti-DNA HRP Antibody** in **1X ELISA Buffer**.
   For Example: Add 20 µl of **Anti-DNA HRP Antibody** 2 ml **1X ELISA Buffer**. This is enough antibody mix for 20 wells.
3. Add 100 µl of antibody mix to each well. Cover the plate with foil and incubate at 37°C for 30 minutes.

Color Development:

1. Discard buffer from the wells of the plate. Wash each well with 200 µl of **1X ELISA Buffer**. Remove all liquid from each well by tapping out excess liquid onto a paper towel. Repeat this wash step 2 more times.
2. Add 100 µl of **1X Developer** to each well and allow color to develop at room temperature for 10 to 60 minutes.
3. Use an ELISA plate reader to measure the well absorbance at 405-450 nm.

Notes:

* For more information regarding 5-hmC detection and quantification using the **Control DNA Set** refer to Appendix A, page 5.

[1]The strips of wells that are not used should be stored in a clean, dry, dark place for use at a later date.

[2] Adding 100 µl anti-5-hmC pAb diluted to 1 ng/µl yields 100 ng per well; however 50-400 ng/well 5-hmC pAb can be used to coat wells depending on the DNA sample being detected.

[3]If the concentration of sample DNA is too high, pre-dilution of DNA in 1X ELISA Buffer down to 20-50 ng/µl prior to denaturation, is recommended.

[4]DNA samples will remain single stranded on ice until dilutions are prepared.

[5]Adding 100 µl of 1 ng/ µl DNA yields a final amount of 100 ng /well; however, 25-200 ng /well DNA can be used with this assay.

Notes:

[1] The **Controls** should always be included together with the samples for every experiment.

Appendix A – Generation of a Standard Curve with the Control DNA Set

For 5-hmC Detection:

Relative levels of 5-hmC in DNA can be determined by comparing the absorbance of samples to **Control A** (0%) serving as a negative control and **Control E** (0.55%) as a positive control[1]. Since the percent 5-hmC content is provided for all controls (Table 1, below), any of the **Controls** can be included to approximate the relative levels of 5-hmC in DNA.

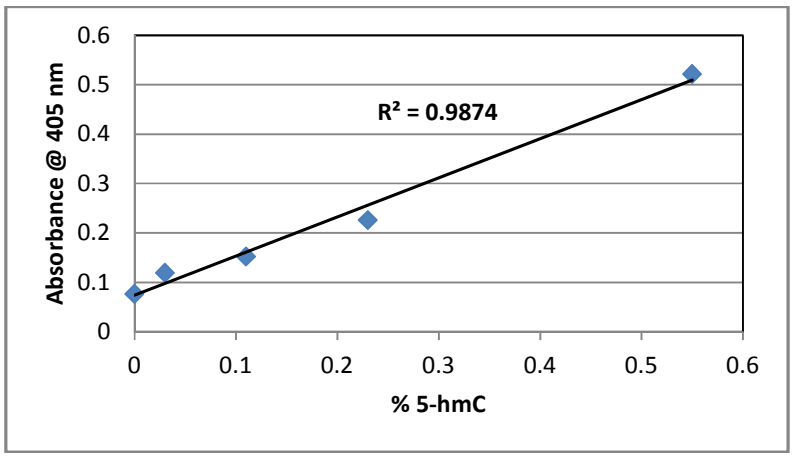
For 5-hmC Quantification:

To quantitate the 5-hmC percentage in a DNA sample, a standard curve[1] must be generated using all the provided **Controls**. Plot the **Control** data as absorbance (Y-axis) vs. percent 5-hmC (X-axis) and use the linear regression (equation below) to determine the "% 5-hmC" for the DNA samples (unknowns).

$$\textbf{\% 5-hmC} = \frac{\textbf{(absorbance} - \textbf{y-intercept)}}{\textbf{Slope}}$$

Table 1. Controls 1-5 and corresponding percent (%) 5-hydroxymethylcytosine.

Control DNA Set (100 ng/µl)	% 5-hmC
Control A	0 %
Control B	0.03 %
Control C	0.12 %
Control D	0.23 %
Control E	0.55 %



An example of a standard curve generated with the Control DNA Set. A standard curve was constructed from the absorbance (405 nm) values of **Controls A-E** (Table 1). The % 5-hmC in any samples is calculated using the equation of the line as shown above.

Ordering Information

Product Description	Catalog No.	Kit Size
Quest 5-hmC™ DNA ELISA Kit	D5425 D5426	1 x 96 wells 2 x 96 wells

For Individual Sale	Catalog No.	Amount
Coating Buffer	D5425-1-15 D5425-1-30	15 ml 30 ml
10X ELISA Buffer	D5425-2-30 D5425-2-60	30 ml 60 ml
Anti-5-Hydroxymethylcytosine Polyclonal Antibody (1 mg/ml)	A4001-25 A4001-50	25 µl 50 µl
Anti-DNA HRP Antibody (100X)	D5425-3-100 D5425-3-200	100 µl 200 µl
HRP Developer	D5425-4-15 D5425-4-30	15 ml 30 ml
Control DNA Set	D5425-5-C	5 × 40 µl
Control A (100 ng/µl)	D5425-5-1	40 µl
Control B (100 ng/µl)	D5425-5-2	40 µl
Control C (100 ng/µl)	D5425-5-3	40 µl
Control D (100 ng/µl)	D5425-5-4	40 µl
Control E (100 ng/µl)	D5425-5-5	40 µl
96-well ELISA Plate (12 x 8-well Strips)	C2020	1 plate

Related Products for 5-hmC Analysis:

Product Name	Size	Catalog No.
Quest 5-hmC™ DNA Enrichment Kit	25 Preps. 50 Preps.	D5420 D5421
Quest 5-hmC Detection Kit™	25 Preps. 50 Preps.	D5410 D5411
Quest 5-hmC Detection Kit™-Lite	25 Preps. 50 Preps.	D5415 D5416
Quest*Taq*™ PreMix	50 Rxns. 200 Rxns.	E2050 E2051
Human Matched DNA Set	2 x 5 µg	D5018
Mouse ^{5hm}C & ^{5m}C DNA Set	4 x 5 µg	D5019
5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set	3 x 2 µg	D5405
DNA Degradase™	500 units 2,000 units	E2016 E2017
DNA Degradase Plus™	250 units 1,000 units	E2020 E2021
5-hmC Glucosyltransferase	100 units 200 units	E2026 E2027
5-Hydroxymethyl dCTP [100 mM]	10 µmol	D1045
5-Hydroxymethylcytosine dNTP Mix [10 mM]	2.5 µmol	D1040
5-Methyl dCTP [10 mM]	1 µmol	D1035
5-Methylcytosine dNTP Mix [10 mM]	2.5 µmol	D1030

Additional Products for Epigenetics Research:

Product Name	Size	Catalog No.
OneStep qMethyl™ Kit	1 x 96	D5310
OneStep qMethyl™-Lite	1 x 96	D5311
Zymo*Taq*™ DNA Polymerase	50 200	E2001 E2002
Zymo*Taq*™ PreMix	50 200	E2003 E2004
EZ DNA Methylation™ Kit	50 200 2 x 96 2 x 96	D5001 D5002 D5003 D5004
EZ DNA Methylation-Gold™ Kit	50 200 2 x 96 2 x 96	D5005 D5006 D5007 D5008
EZ DNA Methylation-Direct™ Kit	50 200 2 x 96 2 x 96	D5020 D5021 D5022 D5023
EZ DNA Methylation-Startup™ Kit	1 Kit	D5024
EZ Bisulfite DNA Clean-up Kit™	50 200 2 x 96 2 x 96	D5025 D5026 D5027 D5028
Universal Methylated DNA Standard	1 set	D5010
Universal Methylated Human DNA Standard	1 set	D5011
Universal Methylated Mouse DNA Standard	1 set	D5012
Human HCT116 DKO Methylation Standards	1 set	D5014
Human HCT116 DKO Non-methylated DNA Standard	5 µg	D5014-1
Human HCT116 DKO Methylated DNA Standard	5 µg	D5014-2
Bisulfite Converted Universal Methylated Human DNA Standard	1 set	D5015
E. coli Non-methylated Genomic DNA	5 µg	D5016
Methylated-DNA IP Kit	10	D5101
ChIP DNA Clean & Concentrator™	50 50	D5201 D5205
Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)	50 µg 200 µg	A3001-50 A3001-200
CpG Methylase (M.SssI)	200 units 400 units	E2010 E2011

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