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.

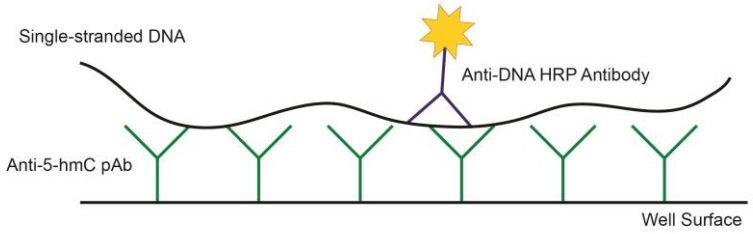
Specifications

- **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 (with 405 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.

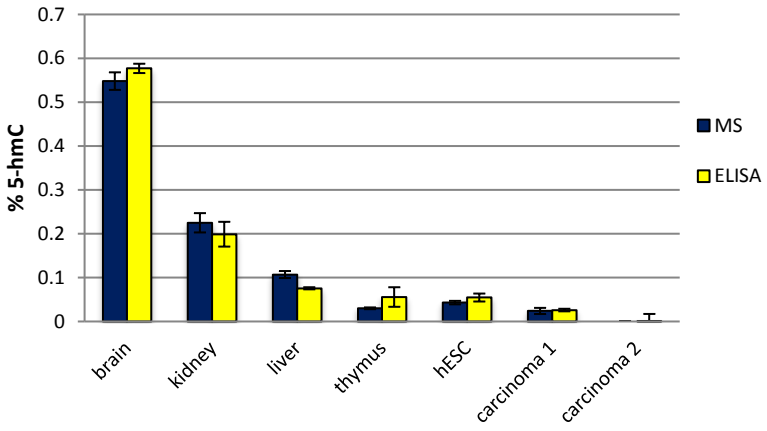
Product Description

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 denatured (single-stranded) DNA/well. Depending on your experimental design, the amount of input DNA can range from 25-200 ng/well. The Control DNA should be assayed at the same concentration as the sample DNA.

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. Add 100 ng of each DNA to a PCR tube and bring the final volume to 100 µl with **1X ELISA Buffer**[3].
    a. For example, add 1 µl **Control A** (100 ng/µl) to 99 µl of **1X ELISA Buffer**.
2. Denature the DNA at 98°C for 5 minutes in a thermocycler. Immediately transfer samples to ice for 10 minutes.
3. Discard buffer from the wells of the plate. Remove all liquid from each well by tapping out excess liquid onto a paper towel.
4. Add 100 µl of the denatured sample and control 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. Dilute **Anti-DNA HRP Antibody (100X)** in **1X ELISA Buffer** to final 1X.
    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 **HRP 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 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] Do not exceed 100 µl final volume in each PCR tube. If denaturing duplicate Control and sample DNAs in same tube (recommended), bring the final volume to 200 µl after denaturing step.

[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] **Control DNA** should always be included together with the samples for every assay to ensure proper functionality.

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.

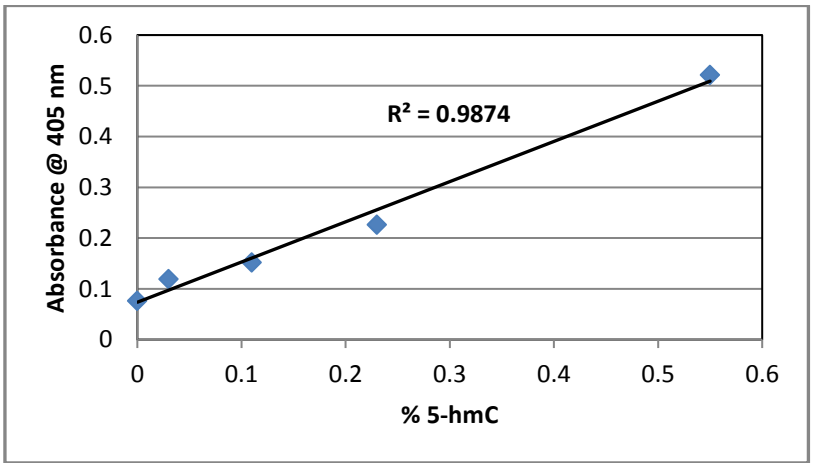
For 5-hmC Quantification:

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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