Product Manual

5-Hydroxymethylcytosine DNA ELISA Kit

Product #: ADI-900- 225

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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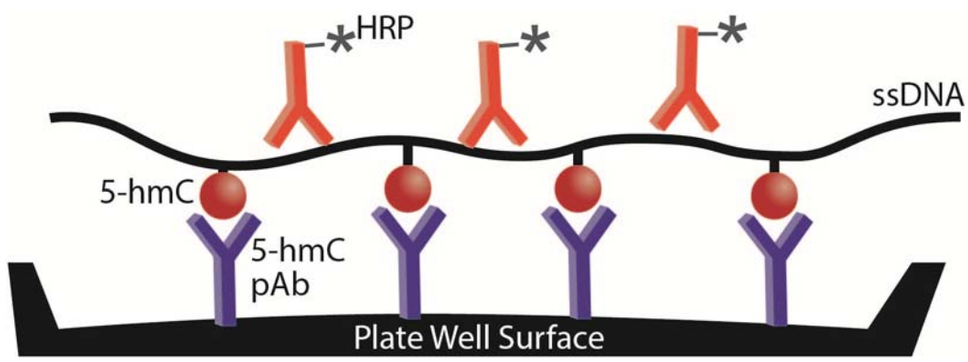
PRODUCT DESCRIPTION

The **5-Hydroxymethylcytosine DNA ELISA kit** is used to accurately quantify the percent 5-hydroxymethlycytosine (5-hmC) DNA in a species independent manner is a variety of DNA samples including intact vertebrate, plant, and microbial genomic DNA, as well as enzyme-digested and mechanically sheared fragments. This highly sensitive ELISA kit allows you to fully quantify results in less than 3 hours with results correlating to alternative methods like mass spectrometry analysis (e.g. LC-MS/MS-MRM). The detection limit is <0.02% 5-hmC DNA per 100ng input DNA, which is ideal for detection of even the smallest percentage of 5-hmC in DNA**.** Sample preparation is minimal and the well-established sandwich ELISA protocol is simple and user-friendly. This ELISA kit 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 96-well format is ideal for high-throughput global 5-hmC DNA detection as well as tissue-specific quantitation, high-throughput compound screening, and more.



The workflow of the 5-Hydroxymethylcytosine DNA ELISA kit utilizes a 5-hmC pAb coated to the bottom of a plate well surfaces. Single stranded 5-hmC-containing DNA (ssDNA) binds to 5-hmC pAb's which is then recognized by a conjugate DNA HRP-Ab. Addition of HRP developer will produce a greenish-blue color in the wells containing 5-hmC DNA.

MATERIALS SUPPLIED

	Size	Storage Temperature
Plate coating buffer	15 ml	4 °C
10X ELISA buffer	30 ml	4 °C
5-hmC pAb (1mg/ml)	25µl	-20 °C
Conjugate DNA HRP-Ab (100X)	100µl	-80 °C
HRP developer	15 ml	4 °C
Control DNA Set (5 Controls)	5 × 40µl	-20 °C
1x96-well plate	1 plate	Room Temp.

NOTE: The integrity of kit components is guaranteed for up to six (6) months from date of purchase, and the conjugate DNA HRP antibody can be stored at -20°C for 1 week.

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 plate coating buffer, pH 9.6, is ready for use and is stable at room temperature or 4°C for extended periods of time. The conjugate DNA HRP-Ab 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. The HRP developer is also ready for use and should be stored at 4°C. For more rapid color development, bring the HRP developer to room temperature before adding to the wells of the ELISA plate.

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 100ng of single-stranded DNA/well. Depending on your experimental design, the amount of input DNA can range from 25-200ng/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 100ng/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).

PROTOCOL

It is recommended that samples and controls be assayed in duplicate for accurate 5-hmC detection in DNA. Please read the entire protocol before proceeding.

Preparing the DNA in the plate -- coating steps

1. Remove the amount of 8-well strips required to assay samples and standards. The strips of wells that are not used should be stored in a clean, dry, dark place for use at a later date.
2. Dilute 5-hmC pAb (1mg/ml) to 1ng/µl in plate coating buffer.
3. Add 100µl/well of the diluted anti-5-hmC pAb. Cover the plate with foil to prevent evaporation and incubate at 37°C for 1 hour. Adding 100µl anti-5-hmC pAb diluted to 1ng/µl yields 100ng per well; however 50-400ng/well 5-hmC pAb can be used to coat wells depending on the DNA sample being detected.

Washing and blocking steps

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

Adding DNA and binding steps

1. Denature the sample and control DNAs at 98°C for 5 minutes in a thermocycler. If the concentration of sample DNA is too high, pre-dilution of DNA in 1X ELISA Buffer down to 20-50ng/µl prior to denaturation, is recommended.
2. Immediately transfer samples to ice for 10 minutes. For DNA samples will remain single stranded on ice until dilutions are prepared.
3. Dilute single-stranded samples and control DNAs to a final concentration 1ng/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 100µl of the diluted sample and standard DNAs to each well. Cover the plate with foil and incubate at 37°C for 1 hour. Adding 100µl of 1ng/µl DNA yields a final amount of 100ng /well; however, 25-200ng /well DNA can be used with this assay.

Adding conjugate DNA HRP-Ab steps

1. Discard buffer from the wells of the plate.
2. Wash each well with 200µl of 1X ELISA buffer.
3. Remove all liquid from each well by tapping out excess liquid onto a paper towel.
4. Repeat this wash step 2 more times.
5. Prepare a 1:100 final dilution of conjugate DNA HRP-Ab in 1X ELISA Buffer.

   For Example: Add 20µl of conjugate DNA HRP-Ab 2 ml 1X ELISA Buffer. This is enough antibody mix for 20 wells.
3. Add 100µl of antibody mix to each well.
4. Cover the plate with foil.
5. Incubate at 37°C for 30 minutes.

Developing the color and reading steps

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

APPENDIX

Standard curve creation with the control DNA set

The controls should always be included together with the samples for every experiment.

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. Since the percent 5-hmC content is provided for all controls (see table below), any of the controls can be included to approximate the relative levels of 5-hmC in DNA. The controls should always be included together with the samples for every experiment.

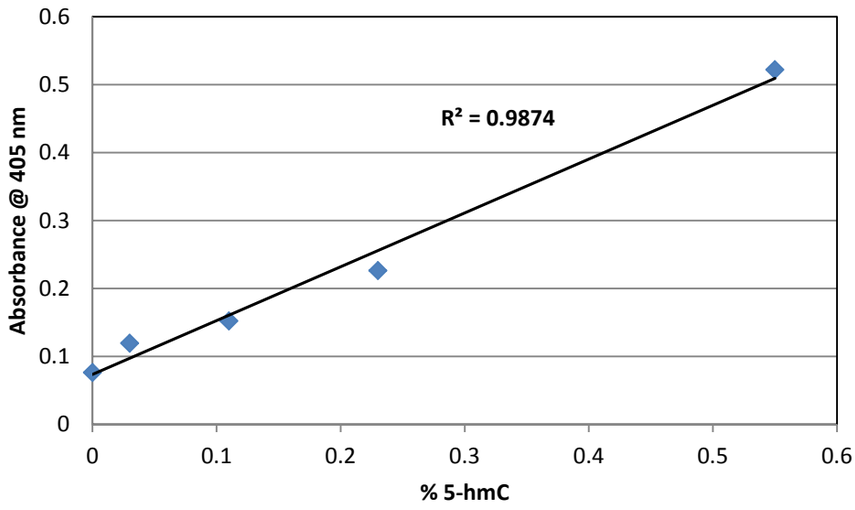
5-hmC Quantification

To quantitate the 5-hmC percentage in a DNA sample, a standard curve must be generated using all the provided controls. The controls should always be included together with the samples for every experiment 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).

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

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

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



A standard curve was constructed from the absorbance (405nm) values of DNA controls A-E (see table above). The % 5-hmC in any samples is calculated using the equation of the line as shown above.

NOTES

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