SuperSense™ Methylated DNA Quantification Kit

Base Catalog # P-1021

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *SuperSense*™ Methylated DNA Quantification Kit is suitable for detecting global DNA methylation status using genomic DNA isolated from any species such as mammal, plant, fungus, bacteria and virus in a variety of forms including cultured cells, fresh and frozen tissues, paraffin-embedded tissues, plasma/serum samples, and body fluid samples, etc.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com
© EpigenTek Group Inc. All rights reserved. Products are for research use only.

KIT CONTENTS

Components	48 samples P-1021-48	96 samples P-1021-96
SA1 (10X Wash Buffer)	15 ml	30 ml
SA2 (DNA Binding Solution)	1.5 ml	3 ml
SA3 (Positive Control, 100 µg/ml)**	10 µl	20 µl
SA4 (Block Solution)	10 ml	20 ml
SA5 (Capture Antibody, 1000 µg/ml)*	5 µl	8 µl
SA6 (Labeling Solution)*	10 µl	20 µl
SA7 (Fluoro Developer)*	12 µl	24 µl
SA8 (Fluoro Enhancer)*	12 µl	24 µl
SA9 (Fluoro Dilution)	4 ml	8 ml
Negative Control DNA (20 ng/µl)	10 µl	20 µl
8-Well Assay Strips (with Frame)	6	12

** For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.*
*** This control is synthesized polynucleotide methylated at every 5-cytosine.*

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **SA3**, **SA6**, **SA7**, and **Negative Control DNA** at –20°C away from light; (2) Store **SA1**, **SA4**, **SA5**, **SA8**, and **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: *Check if wash buffer, SA1, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- Orbital shaker
- Pipettes and pipette tips
- Fluorescence microplate reader
- 1.5 ml microcentrifuge tubes

GENERAL PRODUCT INFORMATION

Quality Control: EpigenTek guarantees the performance of all products in the manner described in our product instructions.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com
© EpigenTek Group Inc. All rights reserved. Products are for research use only.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The *SuperSense*™ Methylated DNA Quantification Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The *SuperSense*™ kits and methods of use contain proprietary technologies by EpigenTek. *SuperSense*™ is a trademark of EpigenTek Group Inc.

A BRIEF OVERVIEW

Epigenetic alterations of genomic DNA play a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer a methyl group from S-adenosyl-L-methionine to the fifth carbon position of the cytosines. Region-specific DNA methylation is mainly found in 5'-CpG-3'dinucleotides within the promoters or in the first exon of genes, which is an important pathway for the repression of gene transcription in diseased cells. Global DNA hypomethylation is likely caused by methyl-deficiency due to a variety of environmental influences, and has been proposed as a molecular marker in multiple biological processes such as cancer. It has been well demonstrated that the decrease in global DNA methylation is one of the most important characteristics of cancer. Thus, the quantification of global methylation in cancer cells could provide very useful information for detection and analysis of this disease.

However, quantification of methylated DNA in very small amounts of tissue or cell samples may be difficult, as sufficient DNA cannot be isolated from these samples. It is common that only minute amounts of tissue or cell samples can be available in biomedical research, high throughput biomarker/drug screening, and pathological diagnosis. These kinds of samples may include tissue biopsy, micro-dissection samples, cells contained in body fluids, cells cultured in 96 and 384 well plates, and early embryonic cells/oocytes.

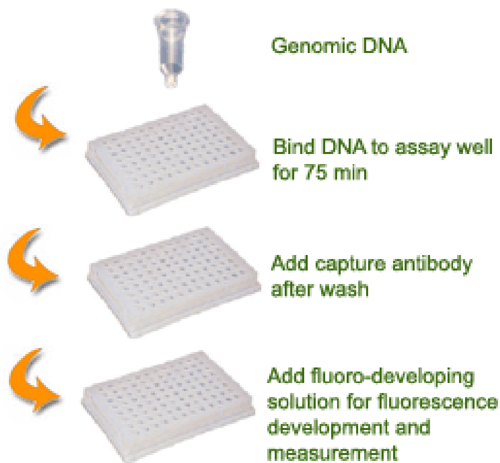
To address this issue, EpigenTek has designed the *SuperSense*™ Methylated DNA Quantification Kit that is suitable for quantifying methylated DNA using small amounts of DNA samples. This kit has the following advantages:

- Fluorescence-based assay with easy-to-follow steps for convenience and speed. The whole procedure can be finished within 2 hours and 30 minutes.
- High sensitivity, of which detection limit can be as low as 50 pg of methylated DNA with the input DNA being as low as 2 ng.
- Universal positive control, which is suitable for quantifying methylated DNA from any species.

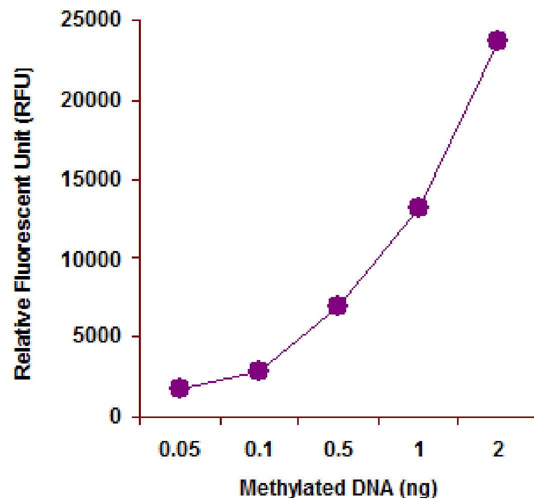
110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com
© EpigenTek Group Inc. All rights reserved. Products are for research use only.

PRINCIPLE & PROCEDURE

The *SuperSense*™ Methylated DNA Quantification Kit contains all reagents required for fluorescent quantification of global DNA methylation. In this assay, DNA is immobilized to the strip well specifically coated with a DNA affinity substance. The methylated DNA can be recognized with an anti-5-methylcytosine antibody. The ratio or amount of methylated DNA, which is proportional to fluorescence intensity, can then be fluorometrically quantified.



Schematic Procedure for Using the *SuperSense*™ Methylated DNA Quantification Kit



Quantification of Methylated DNA

PROTOCOL

1. Prepare DNA by using your own successful method. For your convenience and the best results, EpigenTek offers a series of DNA isolation kits which are optimized for extracting DNA from cultured cells, tissues, body fluids, and paraffin sections.
2. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Dilute SA1 with distilled water (pH 7.2-7.5) at a 1:10 ratio (ex: 1 ml of SA1 + 9 ml distilled water).
3. For samples, add 28 µl of SA2 solution into the well, followed by adding 2 µl (10-100 ng) of sample DNA. For the positive control, dilute SA3 with SA2 at a 1:40 ratio (ex: 1 µl of SA3 + 39 µl of SA2). Add 28 µl of SA2 into the strip well, followed by adding 2 µl of the **diluted** SA3 solution (5 ng/well). Optionally, dilute SA3 to 0.05-5 ng/µl, respectively with SA2. Add 28 µl of SA2 into the strip well, followed by adding 2 µl of each **diluted** SA3 solution to generate a standard curve [4-6 points. Ex: 0.1, 0.5, 1, 2, 5, and 10 ng/well]. For negative control*, add 28 µl of SA2, followed by adding 2 µl of Negative Control DNA into the wells. Shake the plate frame to allow the solution to cover the whole surface of strip well bottom. Incubate the strip wells at 65°C (with no humidity) for 45 minutes to evaporate the solution and dry the wells.

   Note: *Occasionally, the non-evaporated solution may be gathered along the edges at the bottom of the well. Make sure the well is completely dry by slightly tilting the well and aspirating against the edge with a P-10 or P-20 pipette. If there is still residue solution, extend incubation time for an*

© EpigenTek Group Inc. All rights reserved. Products are for research use only.

additional 5-10 minutes at 65°C to dry the well. It is normal if white salt precipitates are seen in the wells, which will eventually be washed out in Step 5.

**Required for background, cannot be substituted with DNA-less control.*

4. Add 150 µl of **SA4** to each dried well. Incubate at 37°C for 30 minutes.
5. Aspirate and wash each well with 150 µl of **diluted SA1** two times.
6. Dilute **SA5** (at a 1:1000 ratio) to 1 µg/ml with **diluted SA1**. Add 50 µl of **diluted SA5** to each well and incubate at room temperature for 60 minutes.
7. Aspirate and wash each well with 150 µl of **diluted SA1** four times.
8. Dilute **SA6** (at a 1:1000-1: 2000 ratio) with **diluted SA1**. Add 50 µl of **diluted SA6** to each well and incubate at room temperature for 30 minutes.
9. Aspirate and wash each well with 150 µl of **diluted SA1** 5-6 times.
10. Prepare the **Fluoro-Development Solution** by adding 1 µl of **SA7** and 1 µl of **SA8** into each 500 µl of **SA9**. Add 50 µl of the **Fluoro-Development Solution** into the wells and incubate at room temperature for 2-4 minutes away from light. The color in the standard wells containing the higher concentrations may turn slightly pink during this period. Measure and read fluorescence on a fluorescence microplate reader at $530_{EX}/590_{EM}$ nm.

    Note: *If the strip well frame does not fit the fluorescence reader, transfer the solution to a standard 96-well microplate and read fluorescence at $530_{EX}/590_{EM}$ nm.*

11. Calculation of DNA methylation.

    For simple calculation, use the following formula:

$$\text{Methylation \%} = \frac{\text{RFU (sample} - \text{NC*)/X}}{\text{RFU (positive control} - \text{NC)} \times 10} \times 100\%$$

Here, the amount of the positive control is 5 ng and sample DNA is 50 ng.

*X is GC content of any species DNA. *NC: negative control.*

For example: GC contents is 41% for human genomic DNA , 42% for mouse and rat, 35% for A thaliana, 38% for yeast, respectively.

*For accurate calculation, plot RFU value versus amount of **SA3** and determine the slope as RFU/ng, then calculate the amount of methylated DNA using the following formula:*

$$\text{Methyl DNA (ng)} = \frac{\text{RFU (sample} - \text{NC)}}{\text{Slope}}$$

$$\text{Methylation \%} = \frac{\text{Methyl DNA amount/X}}{\text{Sample DNA amount added}} \times 100\%$$

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com
© EpigenTek Group Inc. All rights reserved. Products are for research use only.

TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples

Problem	Solution
Reagents are added incorrectly.	Check if reagents are added in order and if any steps of the procedure may have been omitted by mistake.
The well is not completely dried.	Ensure the well is incubated with no humidity and dry before adding the block buffer.
The well is incorrectly washed before DNA coating.	Ensure the well is not washed before adding control DNA.
Incubation time and temperature is incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.

No Signal or Very Weak Signal for Only the Positive Control

Problem	Solution
The positive control DNA is insufficiently added to the well.	Mix the control DNA thoroughly before adding to the well. Ensure a sufficient amount of control DNA is added.
The control DNA is degraded due to incorrect storage.	Ensure the control DNA is properly stored according to the storage instructions and has not expired.

High Background Present for the Negative Control

Problem	Solution
The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Contaminated by sample or positive control DNA.	Ensure the well is not contaminated from adding sample or positive control DNA accidentally.
No or insufficient blocking.	Ensure the well is properly blocked with SA4.
Overdevelopment.	Decrease the development time at step 10.

RELATED PRODUCTS

P-1014B Methylamp™ Global DNA Methylation Quantification Ultra Kit

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com
© EpigenTek Group Inc. All rights reserved. Products are for research use only.