Human Septin9 Gene Methylation Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

For Research Use Only

Instruction Version: S2.0 Revision Date: January 2024

Product Name

Human Septin9 Gene Methylation Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

20 Tests/Kit

Intended Use

This kit uses multiplex fluorescence PCR amplification technology to qualitatively detect methylation status of Septin9 gene from cfDNA isolated from peripheral blood plasma. The assay is indicated only for the validation of Septin9 gene methylation status. This kit is intended for research use only. .

Septin9 is a member of Septin9 family, which is involved in cell division, cell polarization, cell exocytosis, cell migration and cell repair. etc. the studies revealed that the high methylation of Septin9 gene is tightly associated with the development of colorectal cancer. Detection of methylation status of Septin9 derived from peripheral blood plasma would contribute to diagnosis of colorectal cancer and improve survival rate of patients effectively.

Technological Principles

This kit uses sulfite to modify and transform genomic DNA to convert unmethylated cytosine into uracil, while methylated cytosine will not change. For the difference mentioned above, this kit uses the transformed genome sequence of transformed Septin9 as the template to design ARMS primers and fluorescent probes for specific methylation sites, and the transformed β-actin sequence of human genome house-keeping gene as internal control template to design internal control primers and fluorescent probes. For product analysis, the use of fluorescently labeled probe real-time tracking analysis makes the detection method automatic. The methylation of special sites in Septin9 gene can be detected by the kit with high specificity and high sensitivity on the real-time PCR platform. When analyzing the results, the FAM signal indicates the Septin9 gene methylation status and the HEX (VIC) signal indicates the internal control.

Kit Contents

Reaction reagents are pre-loaded in 8-tube strips; each strip detects two samples, and each PCR reaction tube contains specific primers, fluorescent probes, dNTPs, $MgCl_2$, etc. Tube 1 and tube 5 contains external control detection reagents, which is indicated by FAM signal. Tube 2-3 and tube 6-7 are intended for the detection of the Septin9 gene methylation status, which are indicated by FAM signal. Tube 4 and tube 8 contains internal control detection reagents, which is indicated by HEX (VIC) signal. Internal control and external control are used as quality control of the reagents, DNA quality and operation.

Table 1. Kit Contents

Content Name	Components	Volume	Quantity
SPT9 8-Tube Strips	Primers, probe, $MgCl_2$, dNTPs	45 μL	12 strips
SPT9 Taq Polymerase	Taq DNA polymerase	35 μL	1 tube
SPT9 Positive Control	Positive plasmid DNA, internal control plasmid DNA	100 μL	1 tube
SPT9 Negative Control	Nuclease-Free water	250 μL	1 tube

Note: The contents of different batches cannot be mixed.

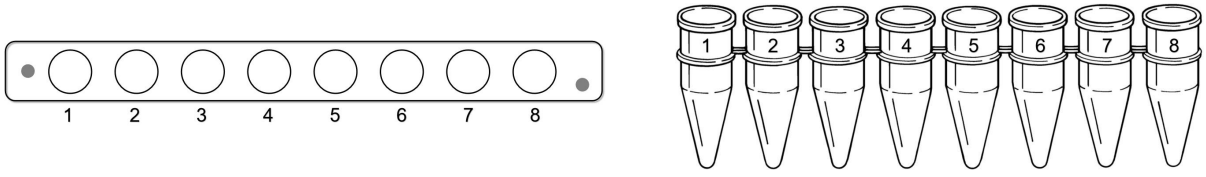

Figure 1. Tube Sequence of 8-Tube Strip

Note: The reaction solutions are pre-loaded in 8-tube strips, as shown in Figure 1. There are two different models on the left and right, which are 1, 2, 3, 4, 5, 6, 7 and 8 tubes from left to right.

Additional required Equipment and Materials

1. It is recommended to use the Nucleic Acid Extraction Kit (Plasma DNA) from Xiamen Spacegen Co., Ltd. for nucleic acid extraction.
2. It is recommended to use the Genomic DNA Bisulfite Modification Kit from Xiamen Spacegen Co., Ltd. for DNA modification transformation.
3. Nuclease-Free water.
4. Aerosol filter pipette tips.

Transportation, Stability and Storage

1. Storage Condition. Store the kit away from light at -15℃ to -25℃, valid for 9 months. Once opened, reagents can be stored in their original packaging at -15℃ to -25℃ until the stated expiration date shown on the packaging. Repeated thawing and freezing should be avoided. Do not exceed a maximum of 5 freeze-thaw cycles.
2. Transportation Condition. The kit should be transported at low temperature, with transporting time less than one week and transporting temperature lower than 25℃.
3. Check labels for production date and expiration date of the kit.

Compatible PCR Instruments

ABI7500, ABI7300, ABI StepOne Plus, LightCycler480, Bio-Rad CFX96, etc.

For ABI instruments, the probe mode setting as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.

Specimen Material

1. Recommended sample types: peripheral blood plasma.
2. Commercialized kit is recommended to extract DNA from the samples. Assess the quality of sample DNA with an microvolume ultraviolet-visible spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.7-2.2. Once the DNA quality or quantity was not in conformity with the above requirements, re-extract DNA with new and/or larger input.
3. Proceed extracted DNA to transforamtion immediately or store the DNA at -15°C to -25°C for no more than 12 months. Freeze-thaw samples no more than 5 times. Proceed transformed DNA to detection immediately or store the DNA at -15°C to -25°C for no more than 1 month. Freeze-thaw samples no more than 3 times.

Experimental Procedure

1. Reagent Preparation

   Prepare **SPT9 8-Tube Strips** and **SPT9 Taq polymerase** according to samples; briefly centrifuge the stripes and Taq polymerase; place them on ice and transfer to the sample processing area; detection of **SPT9 Positive Control** (PC) and **SPT9 Negative Control** (NTC) in each reaction/run are recommended.

2. Sample Processing

   (1) Sample preparation: According to the instructions of the Genomic DNA Bisulfite Modification Kit to modify and transform the tested DNA, the elution volume after transformation is for a minimum of 20 μL, if the volume after elution is less than 20 μL, diluted to 20 μL with Nuclease-Free Water, which is so called tested DNA.

   (2) Template preparation: Respectively add 1 μL **STP9 Taq polymerase** to 20 μL of the tested DNA, PC, and NTC, vortex slightly to mix, then pulse centrifuge.

   (3) Gently remove the cap of 8-tube strip, sequentially add 5 μL of the templates into tubes of each strip, cover the cap carefully.

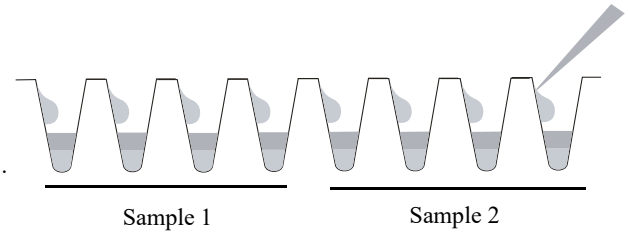

Figure 2. The 8-Tube Stripe Sampling Diagram

3. Amplification

(1) Centrifuge the 8-tube stripes for 10 seconds to collect templates.

(2) Load the 8-tube strips into the real-time PCR instrument; refer to Table 2 for overall arrangement if necessary.

Table 2. Suggested Overall Arrangement

Reagent Name	Reporter Dyes	1	2	3	4	5	6	7	8	9	10	11	12
SPT9-1	FAM	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	Sample15	Sample17	Sample19	Sample21	PC
SPT9-2	FAM	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	Sample15	Sample17	Sample19	Sample21	PC
SPT9-3	FAM	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	Sample15	Sample17	Sample19	Sample21	PC
SPT9-4	HEX	Sample1	Sample3	Sample5	Sample7	Sample9	Sample11	Sample13	Sample15	Sample17	Sample19	Sample21	PC
SPT9-1	FAM	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	Sample16	Sample18	Sample20	Sample22	NTC
SPT9-2	FAM	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	Sample16	Sample18	Sample20	Sample22	NTC
SPT9-3	FAM	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	Sample16	Sample18	Sample20	Sample22	NTC
SPT9-4	HEX	Sample2	Sample4	Sample6	Sample8	Sample10	Sample12	Sample14	Sample16	Sample18	Sample20	Sample22	NTC

(3) Set and run the amplification program as shown in Figure 3.

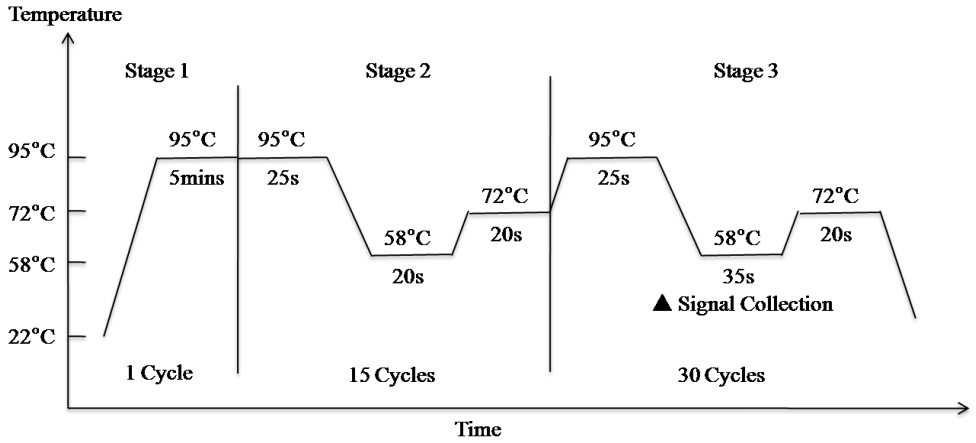

Figure 3. PCR Amplification Procedure

(4) Handle the strips properly after experiment; do not remove the caps in case contamination.

Data Analysis

1. The ΔCt Cut- off value of the methylation detection in this kit was determined as 9 with the assist of ROC curve method.

2. Result Judgment

(1) Ct value: provided by the instrument software or by determining the threshold fluorescence of actual amplification curve.

(2) Result Judgment:

a) When the FAM Ct of SPT9-2 or SPT9-3 is greater than 29, a negative call, or lower than the detection limit of the kit is returned.

b) When the FAM Ct is less than or equals to 29, calculated the ΔCt Cut-off value per the equation below. If the derived ΔCt Cut-off value is less than or equals to 9, a positive call is returned; If the derived ΔCt Cut-off value is greater than 9, a negative call is returned.

Equation: ΔCt Cut-off = Ct (Methylation) – Ct (External)

Ct (Methylation): The FAM Ct of SPT9-2 or SPT9-3.

Ct (External): The FAM Ct of SPT9-1.

Interpretation of Results

1. NTC: There should be no amplification curves of FAM and HEX (VIC); or else, call the result invalid.
2. PC: There should be amplification curves of FAM and HEX (VIC), with the value of Ct is less than or equals to 24. If the Ct value of FAM or HEX (VIC) in any one tube is greater than 24, the value is invalid and retest is recommended.
3. Internal Control: The HEX (VIC) Ct of every sample in tube of internal control (tube 4 or tube 8) should be less than or equals to 24, which must be qualified before proceeding to further analysis; If the HEX (VIC) Ct is greater than 24, that indicates insufficient DNA amount or that sample DNA was contaminated by PCR inhibitor, in this case, it is recommended to re-extract sample DNA for a new detection.
4. External Control: The FAM Ct of every sample in tube of external control (tube 1 or tube 5) should be less than or equals to 24, which must be qualified before proceeding to further analysis; If the FAM Ct is greater than 24, that indicates insufficient DNA amount or that sample DNA was contaminated by PCR inhibitor, in this case, it is recommended to re-extract sample DNA for a new detection.

Limitations of the Kit

1. Negative results could not exclude the existence of Septin9 gene methylation; cases like DNA degradation, or insufficient DNA amount may lead to negative results as well.
2. Situations that may result in false negative or false positive result include but not limit to unreasonable sample collection, transportation, improper experimental operations or environment.
3. The kit is only applicable with the stated sample types and detection system, including specified instruments, DNA extraction and transformation kit, and analytical assay.

Performance Characteristics

1. The kit should be of neat appearance, clear labels, and of no leakage. When unfrozen, the reagents shall be clear, without precipitate.
2. The consistency rates of both positive and negative reference materials are 100%.
3. Repeat the test 10 times for the same precision reference material, the coefficient of variation (CV, %) of the Ct value should be less than 10%.

Warnings and Precautions

1. Please read the instruction carefully in prior to the use of the kit.
2. Avoid repetitively freezing and thawing the reagents in the kit.
3. The results of this kit will be affected by the source, the process of collection, quality, condition of transport, pre-treatment of the sample, as well as the quality of the extracted DNA, model of fluorescence quantitative PCR instrument, operation environment, and the current technological limitation of molecular biology. The factors and variables mentioned above would lead to false positive or false negative test results. Users must be aware of the potential errors and accuracy limitations that may exist during the process of detection.
4. The quality of DNA is crucial, and the quality control of DNA should be performed after extraction; proceed to sample detection immediately or store sample DNA properly at -15℃ to -25℃.
5. Do not substitute any content of the kit; do not mix contents of different batches.
6. Pay special attention to the use of positive control to prevent contamination of reagents or resulting in false positive results.
7. Be cautious of contamination from external DNA; when sampling, always add NTC and sample DNA before positive control; segregate areas for reagent preparation and sample processing; use dedicated pipettes and tips for reagent preparation and template addition, respectively.
8. Sterilize the environment and pipettes with 10% hypochlorous acid, 75% ethyl alcohol, or UV radiation.
9. All the reagents in use have potential hazard. It is recommended wearing proper protective suit and gloves. For first-use of this kit, you may receive training by our technical supports.
10. All samples including positive control in the kit should be considered as potential infectious substances which should be handled carefully.

Symbols

Symbol	Symbol Definition
	Indicates the need for the user to consult the instructions for use.
	Indicates the date when the medical device is manufactured.
	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Indicates the temperature limitation.
	Indicates the date after which the medical device is not to be used.
	This is the correct upright position of the distribution packages for transport or storage.
	Indicates a medical device should be kept dry.
	Indicates a medical device that needs protection from light sources.
	Indicates the medical device manufacturer.

References

1. Devos T, Tetzner R, Model F, et al. Circulating methylated SEPT9 DNA in plasma is a biomarker for colorectal cancer[J]. Clinical Chemistry, 2009, 55(7):1337.
2. Catherine L D, Fabian M, Theo D, et al. DNA methylation biomarkers for blood-based colorectal cancer screening. [J]. Clinical Chemistry, 2008, 54(2):414.
3. Molnar B, Pilarsky C, Habermann J K, et al. Sensitive detection of colorectal cancer in peripheral blood by septin 9 DNA methylation assay. [J]. Plos One, 2008, 3(11): e3759.
4. Warren J D, Wei X, Bunker A M, et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer[J]. Bmc Medicine, 2011, 9(1):133-133.
5. Tóth K, Sipos F, Kalmár A, et al. Detection of Methylated SEPT9 in Plasma Is a Reliable Screening Method for Both Left- and Right-Sided Colon Cancers[J]. Plos One, 2012, 7(9): e46000.
6. Church TR; Wandell M; Lofton-Day C; Mongin SJ; Burger M; Payne SR; Castaños-Vélez E; Blumenstein BA; Rösch T; Osborn N; Snover D; Day RW; Ransohoff DF; PRESEPT Clinical Study Steering Committee, Team I S. Prospective evaluation of methylated SEPT9, in plasma for detection of asymptomatic colorectal cancer[J]. Gut, 2014, 63(2):317-325.

Manufacturer: XIAMEN SPACEGEN CO., LTD.
Address: 4th floor, No.2041 Xizhou Road, Xike Town, Tong'an District, Xiamen 361100, P. R. China
Tel: +86 592 7578317 Fax: +86 592 7578319
E-mail: spacegen@ispacegen.com
Website: http://www.sspacegen.com