CE

PAP-ARMS®

MGMT Gene Methylation Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

Product Name

MGMT Gene Methylation Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

24 Tests/Kit

Intended Use

This kit uses fluorescent PCR amplification technology, extracting DNA from paraffin-embedded tissue sections to qualitatively detect MGMT gene CpG island (hg19: chr10: 131265575, 131265580, 131265586, 131265596, 131265609, 131265614, 131265626) methylated status. This kit is only for verification of the detection performance of the target gene, and the detection results are only for clinical reference.

The O'6-methylguanine-DNA methyltransferase (MGMT protein) encoded by MGMT gene can move away the alkylation-adducts from O'6-methylguanine which may hinder the DNA duplication, in other words, the MGMT can protect the cells during the process. For glioma patients, different frequencies of methylation may always happen in the promoter region of MGMT gene. Although methylation of MGMT gene will reduce the expression level or activity of MGMT protein, the patients with MGMT methylation will be more sensitive to some kinds of tumor drugs. Meanwhile, some researches have shown that, glioma patients with methylation in special genes of MGMT gene may response to temozolomide effectively.

Technological Principles

The genomic DNA should be transformed with the matched DNA Methylation Kit. During the transforming process, unmethylated cytosine (C for short) will be transformed to Uracil (U for short) finally; While for the methylated C, it will remain unchanged since the protection from methyl.

For the difference mentioned above, the kit designs ARMS primers based on the sequence of transformed MGMT sequence. The length of target sequence and internal control (transformed sequence of β-actin gene) is 80 bp and 90 bp, respectively. For product analysis, the use of fluorescently labeled probe real-time tracking analysis makes the detection method automatic. The methylation of special sites in MGMT 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 MGMT gene methylation status and the HEX (or VIC) signal indicates the internal control.

Kit Contents

Reaction reagents are pre-loaded in 8-tube strips; Each tube in every 8-tube strip contains same contents for detection of one sample (Table 1). The FAM signal indicates the MGMT gene methylation status and the HEX (or VIC) signal indicates the internal control.

Table 1. Kit Contents

Content Name	Components	Volume	Quantity
MGMT 8-Tube Strips	Primers, Probes, Mg^{2+}, dNTPs	45 μL	4 strips
MGMT Taq Polymerase	Taq DNA polymerase	15 μL	1 tube
MGMT Positive Control	Positive plasmid DNA, Wild type DNA	50 μL	1 tube
MGMT Negative Control	Nuclease-free purified water	50 μL	1 tube

Note: The contents of different batches cannot be mixed.

Equipment and Reagents Required

1. Nucleic acid extraction kit: commercial nucleic acid extraction kits are recommended;
2. Matched DNA methylation kit, DNA Methylation Kit from Xiamen Spacegen Co, Ltd. is recommended, Cat. No. SPG-DM001;
3. Nuclease-free purified water;

4. DNase-free and RNase-free pipettes and tips.

Transportation, Stability and Storage

1. Storage Condition: Store the kit away from light at -20±5°C, valid for 9 months. Once opened, the kit is stable at - 20±5°C until the stated expiration date. Do not use the reagents after 5 freezing-thawing cycles.
2. Transportation Condition: The kit should be transported in foam cases with ice bags, with transporting time less than one week and transporting temperature lower than 25°C.
3. Check labels for production date and expiration date of the kit.

Compatible PCR Instruments

Stratagene Mx3000P™, ABI7500, ABI7300 Plus, SLAN-96P/SLAN-48P

Note: For ABI instruments, define targets and passive reference as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.

Specimen Material

1. Recommended sample types: FFPE tissues, ensure that at least 30% of the collected pathological tissue were tumor lesions; Choose FFPE samples which have not been stored for more than 2 years.
2. Commercial kits are highly recommended to extract genomic DNA from the above samples. Assess the quality of sample DNA with an ultraviolet spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.7 - 2.2; the concentration should be >25 ng/μL. Once the DNA quantity or quality was not in conformity with the above requirements, re-extract DNA with new and/or larger amount of samples.
3. Proceed extracted DNA to transforamtion immediately or store the DNA at - 20±5°C for no more than 12 months; The cycle of freezing and thawing the extracted DNA should be no more than 5 times;
4. Proceed transformed DNA to detection immediately or store the DNA at - 20±5°C for no more than 1 month; The cycle of freezing and thawing the tranformed DNA should be not more than 3 times.

Experimental Procedure

1. Reagent preparation
   a) Prepare 8-tube stripes and MGMT Taq polymerase per the number of samples; Briefly centrifuge the stripes and Taq polymerase; Place them on ice before transferring to the sample processing area; Detection of MGMT Positive Control (PC) and NTC in each reaction / run are suggested.
2. Sample Processing
   a) Commercialized kit is recommended to extract genomic DNA and the DNA sample is transformed with the matched transformation kit. Then, it was diluted with nuclease-free water to 5 ng/μL, which is so called tested DNA;
   b) Respectively pipet 0.4 μL MGMT Taq polymerase to 5 μL of the tested DNA, or PC / NTC, vortex slightly followed by a brief centrifugation, which is so called template for amplification;
   c) Gently remove the cap of 8-tube strip, sequentially pipet 5 μL of the templates into tubes of each strip, cover the cap carefully.

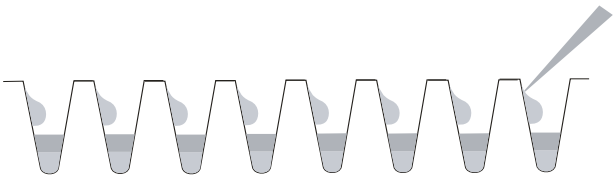

Figure 1. The 8-Tube Strip Sampling Diagram

3. Amplification
   a) Centrifuge the 8-tube stripes for 10 seconds to collect templates;
   b) 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

No.	1	2	3	4	5	6	7	8
A	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	PC	NTC
B	Sample7	Sample8	Sample9	Sample10	Sample11	Sample12	PC	NTC
C	Sample13	Sample14	Sample15	Sample16	Sample17	Sample18	PC	NTC
D	Sample19	Sample20	Sample21	Sample22	Sample23	Sample24	PC	NTC

c) Set and run the amplification program as shown in Figure 2;

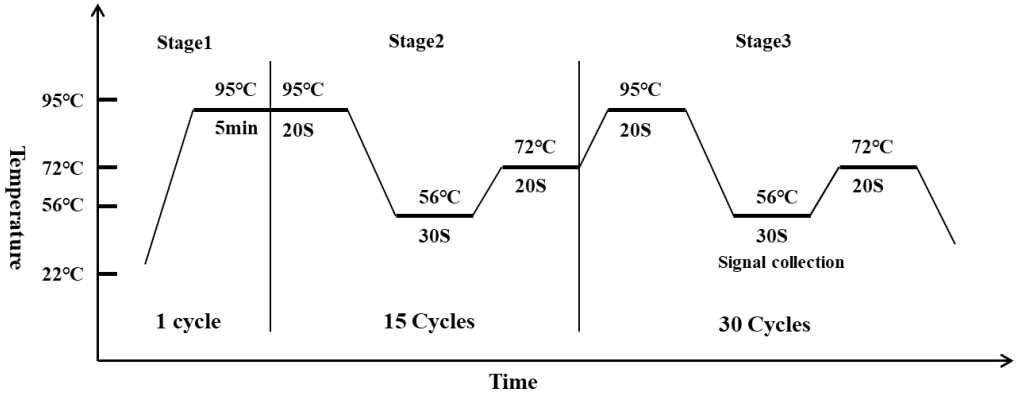

Figure 2. PCR Amplification Procedure

d) Handle the strips properly after experiment; To avoid contamination, do not remove the caps to prevent contamination.

Positive Judgment Value

1. The positive value was determined as 28 in clinical trials with the assist of ROC curve method.
2. Result Judgment
   a) Ct value: Provided by the instrument software or by determining the threshold fluorescence of actual amplification curve;
   b) If the sample FAM channel has no rise in the amplification curve, the sample is negative (or below the detection limit of the kit);
   c) If the FAM channel of the sample has a rising amplification curve and the Ct value is less than 28, the sample is positive;
   d) If the amplification curve of the FAM channel of the sample rises, and the Ct value is $28 \leq Ct < 30$, increase the sample load and retest; After the retest, the Ct value is less than 28, it is judged to be positive; Otherwise, it is judged to be negative (or lower than detection limit of the kit).

Interpretation of Results

1. NTC: There should be no amplification curves of FAM; Or else, call the result invalid. Occasionally, amplification curve of HEX (or VIC) generates, which has no influence on result interpretation.
2. PC: The FAM Ct of PC is always less than 24, but varies among different instruments due to various fluorescence thresholds.
3. Internal Control: Amplification curves of HEX (or VIC) should generate for every sample, the Ct value is ranged from 12 to 20; If the HEX(VIC) Ct value is less than 12, that indicated excessive DNA amount, dilute the transformed DNA for a new detection; If the HEX(VIC) Ct value is more than 20, that indicates insufficient DNA amount or that sample DNA was contaminated by PCR inhibitor, in this case, re-extract sample DNA for a new detection.

Limitation of The Kit

1. Negative results could not exclude the existence of MGMT gene methylation; cases like inadequate tumor cells, DNA degradation or, insufficient DNA amount may lead to negative results as well.
2. Different sampling locations may lead to diverse outcomes due to the heterogeneity of tumor tissues / cells.
3. 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.
4. The kit is only intended for the detection of methylation status of specific MGMT gene sites.

5. The kit is only applicable with the stated sample types and detection system, including specified instruments, DNA extraction and transformation kit, and analytical assay.

Physical Performance

1. The kit should be of neat appearance, clear labels, and of no leakage; When unfrozen, the reagents shall be clear, without sediments.
2. The consistency rates of both positive and negative control reference samples are 100%.
3. The coefficient of variation (CV, %) of 10 Ct values by detecting designated sample for 10 repetitive times should be less than 10%.

Precautions and Warning

1. Please read the instruction carefully in prior to the use of the kit.
2. Avoid repetitively freezing and thawing reagents.
3. Perform quality control of DNA after extraction; Proceed to sample conversion immediately or store sample DNA properly.
4. Do not substitute any content of the kit; Do not mix contents of different batches.
5. Pay special attention to the use of positive control to prevent contamination of reagents or resulting in false positive results.
6. Be cautious of contamination from external DNA; When sampling, always pipet NTC and sample DNAs before positive control; Segregate areas for reagent preparation and sample processing; Use dedicated pipettes and tips for reagent preparation and template addition, respectively.
7. Sterilize the environment and pipettes with 10% hypochlorous acid, 75% ethyl alcohol, or UV radiation.
8. All the reagents in use have potential hazard. Only people who have work permit for PCR laboratories are allowed to use this kit. It is suggested to wear proper protective suit and gloves. For first-use of this kit, you may receive training by our technical supports.
9. All samples including positive control in the kit should be considered potential infectious substances. They should be handled carefully.

Notes

Symbol	Legend
	Indicates the need for the user to consult the instructions for use.
	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Indicates the date when the medical device was manufactured.
	Indicates the manufacturer’s batch code so that the batch or lot can be identified.
	Indicates the temperature limits to which the medical device can be safely exposed.
	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 that needs to be protected from moisture.
	Indicates a medical device that needs protection from light sources.

	Indicates the medical device manufacturer.
	Indicates the authorized representative in the European Community/European Union.
	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC.

References

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2. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. The New England Journal of Medicine, 343(23): 1350-1354, 2000.
3. Hegi ME, Diserens AC, Godard S, et al. Clinical trial substantiates the predictive value of O-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. Clinical Cancer Research, 10(6): 1871-1874, 2004.
4. Bady P, Sciuscio D, Diserens AC, et al. MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. ActaNeuropathologica, 124(4): 547–560, 2012.

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