PAP-ARMS®

MLH1 Gene Methylation Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

Product Name

MLH1 Gene Methylation Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Package Specification

24 Tests/Kit

Intended Use

This kit uses fluorescence PCR amplification technology and the DNA extracted from paraffin embedded tissue sections as the detection sample to qualitatively detect the methylation of MLH1 gene promoter in DNA samples. This kit is only for the detection performance of target genes and the detection results are only for Clinical reference.

Summary

The protein encoded by MLH1 gene is involved in DNA mismatch repair. MLH1 promoter methylation is common in sporadic microsatellite unstable tumors including colorectal cancer and endometrial cancer, which is related to the loss of MLH1 protein expression. It is rarely found in Lynch syndrome (hereditary nonpolyposis colorectal cancer, HNPCC). Therefore, MLH1 promoter methylation analysis can be used to distinguish sporadic and hereditary colorectal cancer and endometrial cancer in microsatellite highly unstable (MSI-H) tumors. In endometrial carcinoma, MLH1 promoter methylation is highly invasive and is associated with poor prognosis.

Detection Principles

This kit uses bisulfite to modify the DNA of tumor tissue samples, in which the unmethylated cytosine is transformed into uracil, while the methylated cytosine remains unchanged. With eight known CpG dinucleotides in the MLH1 promoter as the target, the detection primers and probes were designed to specifically amplify the MLH1 promoter sequence. The probe fluorescence signal was monitored on the real-time PCR platform to detect the methylation of MLH1 promoter. The kit can detect the methylation status of MLH1 gene promoter and the specific site in sample DNA on the real-time PCR platform, with high specificity and sensitivity. In the final result analysis, the detection of MLH1 methylation site is indicated by FAM signal, and the internal control is indicated by HEX (or VIC) signal.

Kit Contents

This kit contains DNA polymerase, positive control, negative control and PCR reaction solution (Table 1). The PCR reaction solution contains MLH1 methylation detection reagent and internal control reagent. The methylation signal is indicated by FAM signal and the internal control is indicated by HEX signal.

Table 1 Composition of The Kit

Component Name	Main Contents	Volume	Quantity
MLH1 reaction solution	Primers, probes, Mg^{2+}, dNTPs	550 μL	2 Tubes
Taq enzyme (MLH1)	Taq DNA polymerase	10 μL	1 Tube
MLH1 positive control	Positive plasmid DNA	50 μL	1 Tube
MLH1 negative control	Purified water	50 μL	1 Tube

Note: Components in kits of different batch numbers cannot be mixed with each other.

Equipment and Reagents Required

1. Commercial nucleic acid extraction reagents;
2. For the DNA methylation kit, it is recommended to use the genomics DNA bisulfite modification reagent of XIAMEN SPACEGEN CO., LTD., CAT No.: SPG-DM001/002;

3. Purified water without DNase and RNase;
4. No DNase and RNase pipette filter element gun tip;
5. Transparent or milky white 8-tube strip.

Transportation, Stability and Storage

1. Storage conditions: The kit shall be stored away from light at - 20 ± 5℃ for 9 months. Storage at - 20 ± 5℃ after opening does not affect the validity period of the product. Do not repeated freezing and thawing of the kit for more than 5 times.
2. Transportation conditions: The kit should be transported at low temperature. The transportation time shall not exceed one week and the transportation temperature shall not higher than 25℃.
3. See the label for the production date and validity period.

Applicable Instruments

1. Stratagene Mx3000P™, ABI7500, ABI7300Plus, SLAN-96P/SLAN-48P.
2. Note:
   a) Use the probe mode setting of ABI instrument, Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE;
   b) Use stratagene Mx3000p™, adjust the signal gain multiplier of FAM channel of the instrument to 1.

Requirements for samples

1. Applicable sample type: Paraffin embedded tissue section sample; After pathological evaluation, paraffin embedded tissue sections should contain at least 30% of tumor cells;
2. It is recommended to use a commercial kit to extract human genomics DNA, and the extracted OD_{260}/OD_{280} value should be in the range of 1.7 - 2.2. If the DNA concentration and purity do not meet the requirements, the samples shall be taken again or the sample size shall be expanded before DNA extraction;
3. The extracted DNA is recommended to be transformed immediately or stored below - 20℃, the storage time shall not exceed 12 months, and the number of repeated freezing and thawing shall not exceed 5 times;
4. The transformed genome is recommended to be used for detection immediately or stored below - 20℃, with a storage time of no more than 1 month and no more than 3 times of repeated freezing and thawing;
5. The preservation time of paraffin embedded pathological tissue or section samples shall not exceed 2 years.

Detection Procedures

1. Reagent Preparation (Reagent reparation area)
   a) Remove the reaction solution from the kit based on sample numbers, then place it on the ice box. After melting, preload 35 μL of the solution into each PCR reaction tube. Cover the lid and place the tube on the ice box. It is recommended to analyze the samples, positive control (PC) and negative control (NTC) at the same time in each PCR reaction.

Note: 8 tube strips are recommended for PCR reaction tubes. Select suitable transparent 8-tube strip or milky white 8-tube strip according to the instruments.

2. Specimen handling (specimen handling area)
   a) It is suggested to use commercial nucleic acid extraction reagent to extract the sample DNA, after modified with bisulfite kit, dilute the transformed genome to 5 ng/μL with purified water without DNase and RNase according to the concentration (ssDNA) measured by micro spectrophotometer, which is the DNA to be tested;
   b) Respectively add 0.25 μL of the Taq enzyme (MLH1) to 5 μL of the tested DNA, MLH1 positive control and negative control, vortex and rapid centrifugal to mix, which is the amplification template;
   c) Add 5 μL of the above amplification templates to each PCR reaction tube according to the example below, carefully cover the lid of PCR reaction tube and move it to the amplification detection area.

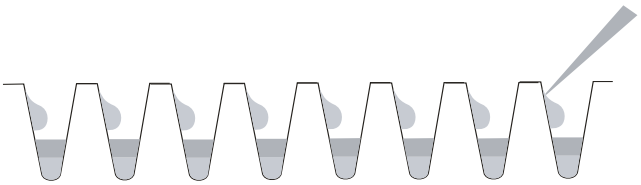

Fig.1 Schematic diagram of 8- tube strip sampling

3. Detection (Amplification detection area)
   a) Quickly centrifuge the 8- tube strip for 10 seconds to collect the added amplification template to the bottom of the reaction tube;
   b) Place the reaction strip into the real-time PCR instrument;
   c) Open the instrument and set the amplification program according to the following figure;

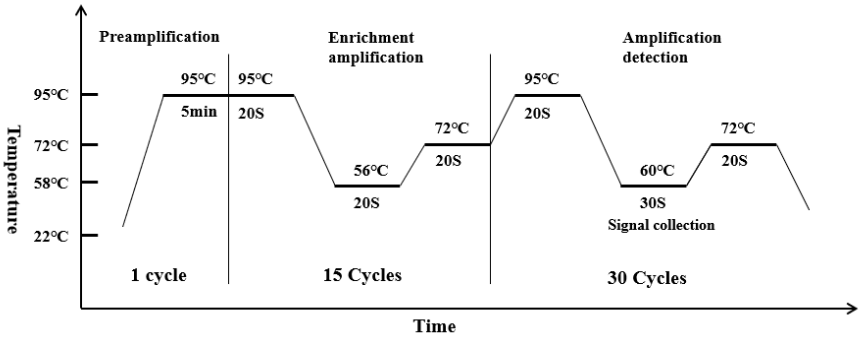

Fig. 2 PCR amplification procedure

   d) Run real-time PCR and save the file;
   e) After the experiment, isolate the PCR reaction strip and do not open the PCR tube cover to prevent pollution.

Interpretation of the Results

1. The FAM signal of negative control (NTC) tube shall have no amplification curve; If the FAM signal rises, the experimental result is invalid; If the HEX (or VIC) signal rises occasionally, it will not affect the judgment of detection results.
2. The Ct value of positive control (PC) is generally < 24, which can fluctuate with the threshold setting of different instruments.
3. The internal control HEX (or VIC) signal of the sample to be tested shall have an amplification curve rising, and the Ct value shall be between 10~18. The next step of analysis shall be carried out after the quality control is qualified. If the Ct value of HEX (or VIC) signal is less than 10, it indicates that the added DNA concentration is too high and should be diluted before move on. If the HEX (or VIC) signal is negative or the Ct value is > 18, it indicates that the added DNA template contains PCR inhibitor or the DNA concentration is too low, so the loading concentration can be increased or the sample should be modified again.

Positive Judgment Value

1. Sample Ct value: Use the Ct value of the amplification curve calculated by the instrument software or determine the inflection point of the amplification curve according to the actual situation to obtain the amplification Ct value.
2. If there is no amplification curve rising in the sample FAM channel, the sample is negative (or lower than the detection limit of the kit);
3. If the amplification curve of the sample FAM channel rises and the Ct value is < 28, the sample is positive;
4. If the sample amplification curve FAM channel rises and Ct value is 28 ≤ Ct < 30, increase the sample loading volume and retest; If the Ct value after retest is less than 28, it is judged to be positive; On the contrary, it is judged as negative (or lower than the detection limit of the kit).

Limitations of Detection Methods

1. Negative results cannot rule out the absence of methylation of MLH1 gene. Negative results can also be caused by too few tumor cells in the sample, excessive degradation of nucleic acid or the concentration of target gene in the amplification reaction system is lower than the detection limit.
2. Tumor tissues (cells) have great heterogeneity, and sampling at different parts can lead to different detection results.
3. Unreasonable sample collection, transfer and treatment, or improper detection operation and the detection environment may lead to false negative or false positive results.
4. This kit is only used for qualitative detection of methylation at specific sites of human MLH1 gene.
5. The detection is limited to the sample type and detection system mentioned in the manual (including applicable models, nucleic acid extraction reagents, detection methods, etc.).

Product Performance

1. The appearance of the kit shall be neat, the mark shall be clear, and there shall be no liquid leakage. After melting, the reagent shall be clear without turbidity and precipitation.
2. The coincidence rate of positive reference was 100%.
3. The coincidence rate of negative reference was 100%.
4. Repeat the test for the same precision reference for 10 times, the test results are positive, and the coefficient of variation (CV %) of Ct value should be less than 10%.

Precautions

1. Please read this manual carefully before the experiment.
2. Avoid repeatedly freezing and thawing the reagent in the kit.
3. The quality of DNA used for detection is very important. After DNA extraction, quality control shall be carried out to determine the extraction quality, and the next test shall be carried out as soon as possible or stored below - 20℃.
4. All reagents in this kit have been specially prepared, and any arbitrary replacement of any reagent may affect the use effect. Components of this kits with different batch numbers cannot be mixed with each other.
5. Note strictly distinguish the use of positive control and reaction reagent to prevent reagent contamination and false positive results.
6. During the experiment, note to prevent foreign DNA pollution and ensure that the positive control operation can be carried out after adding the sample DNA. It is recommended to use separate and special pipette gun and filter head when preparing reaction reagent and adding DNA template. The place where the reaction reagent is prepared shall be isolated from the place where the template is added.
7. After the experiment, the workbench and pipette were treated with 10% hypochlorous acid or 75% alcohol or ultraviolet lamp.
8. All chemicals are potentially dangerous. This kit can only be used by personnel with PCR laboratory certificate. Before using this kit for the first time, the company's technical support should train the operators. During operation, please wear appropriate laboratory work clothes and protective gloves.
9. All test samples and positive controls in the kit should be regarded as infectious substances and should be handled carefully. The used kits are clinical waste and should be properly disposed of.

Index of symbol

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.

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

References

1. Loukovaara M, Pasanen A, Bützow R. Mismatch repair protein and MLH1 methylation status as predictors of response to adjuvant therapy in endometrial cancer. Cancer Med. 2021 Feb; 10(3): 1034-1042. doi: 10.1002/cam4.3691. Epub 2021 Jan 15. PMID: 33449452.
2. Newton K, Jorgensen NM, Wallace AJ, Buchanan DD, Lalloo F, McMahon RF, Hill J, Evans DG. Tumour MLH1 promoter region methylation testing is an effective prescreen for Lynch Syndrome (HNPCC). J Med Genet. 2014 Dec; 51(12): 789-96. doi: 10.1136/jmedgenet-2014-102552. Epub 2014 Oct 3. PMID: 25280751.
3. Zhang YH, Wu HW, Wang J, Liang ZY. Analysis of micro satellite instability in endometroid carcinoma with deficient mismatch repair. Zhonghua Bing Li Xue Za Zhi. 2021 May 8; 50(5): 470-475. Chinese. doi: 10.3760/cma.j.cn112151-20210201-00114. PMID: 33915653.

EC REP

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