Human MYD88 Gene L265P Mutation Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

Product Name

Human MYD88 Gene L265P Mutation Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

20 Tests/Kit

Intended Use

The kit uses multiplex fluorescence PCR amplification technology to qualitatively detect L265P mutation of MYD88 gene in DNA isolated from paraffin-embedded (FFPE) tumor tissue samples from patients with lymphoma. The test results are for research use only.

Myeloid differentiation primary response protein MYD88 is an adaptor protein located in cytoplasm which has two special domains: TIR domain in C tail is involved in homotype interaction with TLR/IL-1R for innate immune response; Death domain in N terminus contributes in signal transduction of apoptosis-related signaling pathways. L265P mutation of MYD88 gene would lead to activation of IL-1 receptor related kinase mediating NF-kB signaling pathway, promoting cell proliferation which is tightly associated with the development of cancer.

Studies revealed that lymphocyte lymphoma/Hua's macroglobulinemia (LPL/WM) patients harboring with L265P somatic mutation of MYD88 is more than 90%, however, MYD88 L265P could also be detected in other small B-cell lymphomas and diffuse large B-cell lymphomas, etc. Consequently, L265P mutation of MYD88 is a notable but no specific marker in the diagnosis of WM.

Technological Principles

The kit uses the sequence of L265P mutation of MYD88 gene as the template to design ARMS primers and fluorescent probes. The length of target gene sequence of each mutant is controlled within 150 bp; The target gene sequences of internal control is conserved sequences on the human genome. For product analysis, the use of fluorescently labeled probe real-time tracking analysis makes the detection method automatic. When analyzing the results, the FAM signal indicates the gene mutation and the HEX (VIC) signal indicates the DNA quality.

Kit Contents

The kit contains MYD88 enzyme mix, MYD88 positive control and MYD88 reaction mix (Table 1). The reaction mix contains reagents of L265P mutation detection and internal control. The FAM signal indicates the L265P mutation and the HEX (VIC) signal indicates the internal control .

Table 1. Kit Contents

Content Name	Components	Volume	Quantity
MYD88 Reaction Mix	Primers, probes, Mg^{2+}, dNTPs	900 µL	1 Tube
MYD88 Enzyme Mix	Taq DNA polymerase, Uracil-N-Glycosylase	13.5 µL	1 Tube
MYD88 Positive Control	Positive plasmid, Reference plasmid	40 µL	1 Tube

Note: The contents of different batches cannot be mixed.

Additional required Equipment and Materials

1. Commercialized nucleic acid extraction kit.
2. Nuclease-Free water.
3. Aerosol-barrier 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,ABI StepOne Plus,QuantStudio5,etc.

1. 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: FFPE tissues stored for no more than 2 years. The biopsies should be fixed with formalin and embedded in paraffin. For resection or surgical biopsies, the recommended tissue input is at least 2×5-micron sections. For coreneedle biopsies, the recommended tissue input is at least 10×5-micron sections.The tissue sample should contain at least 20% tumor cells, otherwise, the tissue samples should be macrodissected and enriched for tumor content.
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, and the concentration is no less than 2 ng/μL. Once the DNA quality or concentration was not in conformity with the above requirements, re-extract DNA with new and/or larger input.
3. Proceed to sample detection or store the DNA at -15℃ to -25℃ for no more than 12 months.

Experimental Procedure

1. Reagent Preparation

   Take out the MYD88 reaction mix and MYD88 enzyme mix from the kit and put them on ice. After the reaction mix melts, take 35 μL of reaction mix and 0.45 μL of enzyme mix according to samples, and pack them into each tube of the 8-tube strips respectively, then cover the cap; place them on ice and transfer to the sample processing area; detection of MYD88 Positive Control (PC) and Negative Control (NTC, Nuclease-Free water) in each reaction/run is recommended.

2. Samples Processing

   (1) Commercialized kit is recommended to extract DNA. Then, dilute sample DNA to 2 ng/μL with Nuclease-Free water according to the concentration determined by microspectrophotometer, and the dilution volume is more than 5 μL, which is so called tested DNA.

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

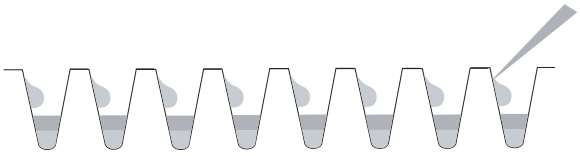

Figure 1. The 8-Tube Strip Sampling Diagram

3. Amplification

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

   (2) Load the 8-tube strips into the real-time PCR instrument.

   (3) Set and run the amplification program as shown in Figure 2.

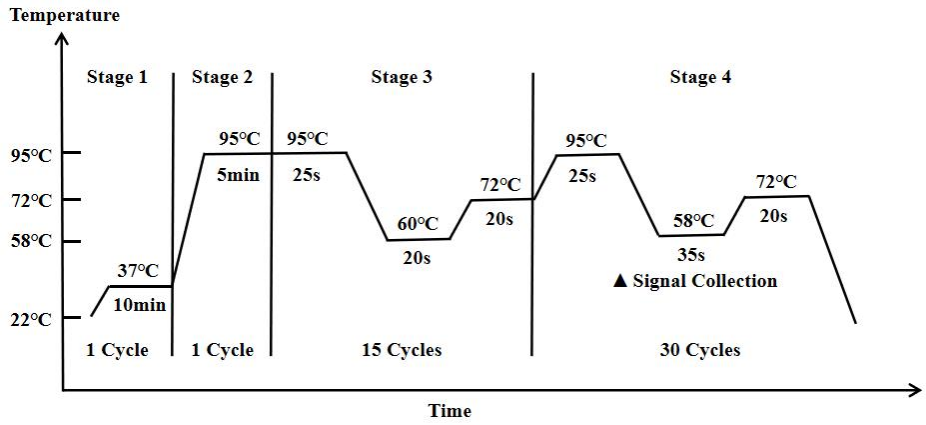

Figure 2. PCR Amplification Procedure

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

Data Analysis

1. The ΔCt-cut-off values in this kit is determined as 10 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) When there are no FAM signal amplification curve rises, a negative call or lower than the detection limit of the kit is returned.
   (3) When the FAM signal amplification curve rises, calculate the ΔCt Cut-off value per the formula below. If the derived ΔCt Cut-off value is less than 10, a positive call is returned; if the derived ΔCt Cut-off value is greater than or equals to 10, a negative call or lower than the detection limit of this kit is returned.
   Formula: ΔCt Cut-off value=Ct (FAM)-Ct (HEX/VIC)

Interpretation of Results

1. NTC: There should be no amplification curves of FAM and HEX (VIC); or else, call the result invalid and retest is recommended.
2. PC: There should be amplification curves of FAM and HEX (VIC), with the value of Ct is less than 24. If the Ct value of FAM or HEX (VIC) is greater than or equals to 24, the value is invalid and retest is recommended.
3. Internal Control: The HEX (VIC) Ct of every sample reaction tube should between 12 and 20, which must be qualified before proceeding to further analysis. If the HEX (VIC) Ct is less than or equals to 12, that indicates excessive DNA concentration, dilute sample DNA for a new detection. If HEX (VIC) Ct is greater than 20,that indicates insufficient DNA concentration 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. The test results of this kit are for scientific research reference only.
2. Negative results could not exclude the existence of MYD88 gene mutation; cases like inadequate tumor cells, DNA degradation, or insufficient DNA amount may lead to negative results as well.
3. Different sampling locations may lead to diverse outcomes due to the heterogeneity of tumor tissues/cells.
4. 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.
5. The kit is only intended for the qualitative detection of L265P mutation of MYD88 gene.
6. The kit is only applicable with the stated sample types and detection system, including specified instruments, DNA extraction 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. The kit allows the detection of 1% of specific gene mutation in 10 ng DNA sample.

4. Repeat the test 10 times for the same precision reference material, all Ct value in FAM and HEX (VIC) of which should be less than 24, and 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. Only people who have work permit for PCR laboratories are allowed to use this kit. 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 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 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.

References

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