PAP-ARMS®

B-raf Gene Mutation Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

Instruction Version: S2.3   Revision Date: September 2023

Product Name

B-raf Gene Mutation Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

20 Tests/Kit

Intended Use

This kit uses multiple fluorescent PCR amplification technology, and uses DNA extracted from paraffin-embedded tissue sections as detection samples to qualitatively detect the B-raf gene (Table 1) in DNA samples. The somatic mutations of V600 provide a clinical reference for drug selection in patients with melanoma, colorectal cancer, thyroid cancer or lung cancer.

B-raf gene is a member of RAF family locates at Chr. 7q34. It encodes serine and threonine protein kinase, and plays an important role in MAPK/ERK signaling pathway. Mutations in the B-raf gene can cause disease in two ways, one is through inheritance, resulting in congenital defects, and the other is through acquired oncogenes that lead to tumorigenesis. More than 40 kinds of B-raf mutations have been detected so far, around 80% of which is V600E mutation in kinase domain (exon 15).

B-raf mutations are frequently (~ 80%) detected in melanoma and thyrophyma, and are seldom detected in lymphoma, rectal carcinoma, liver cancer, breast cancer, ovarian cancer or lung cancer; the mutation rate of B-raf gene in non-small cell lung cancer (NSCLC) is about 3%, and adenocarcinoma accounts for the most.

There are two main types of B-raf mutations, 11% located in the glycine ring on exon 11, such as point mutations in G463, G465, G468, etc., and 89% which occur in exon 15 mutations in the activation region, of which about 92% are located at nucleotide 1799, and the base T is mutated to A (T1799A), resulting in the substitution of its encoded valine by glutamic acid (V600E). Studies have shown that patients with mutations in the B-raf gene have low efficacy in receiving EGFR-TKI drugs, and the V600E mutation of the B-raf gene can cause part of the wild-type K-ras gene type patients not sensitive to EGFR-TKI drug treatment.

Table 1. Somatic Mutations Detected by the Kit

Mutation Name	Changes of Bases	Cosmic ID
V600E1	1799T>A	476
V600K	1798_1799GT>AA(complex)	473
V600E2	1799_1800TG>AA (complex)	475
V600R	1798_1799GT>AG(complex)	474
V600D1	1799_1800TG>AC(complex)	/
V600D2	1799_1800TG>AT(complex)	477

Technological Principles

The kit designs ARMS primers based on the sequence of designated mutation sites. The length of target sequence and internal / external control (conserved sequence of human genome) is < 150 bp and 100 bp, respectively. For product analysis, the use of fluorescently labeled probe real-time tracking analysis makes the detection method automatic. Specific fluorescence probes are fluorescence-marked probes of specified sequences, with the reporter group at 5'-end and the quencher group at 3'-end. When the probe is complete, the fluorescence is quenched between two groups, while during the amplification of specified sequences, the reporter group will be hydrolyzed with the 5'-end exonuclease activity of Taq DNA polymerase, thus separating the two groups, and releasing the specific fluorescence. In other words, amplification of one DNA strand means the formation of one fluorescence molecule, thus synchronizing the accumulation of fluorescence signal with the PCR procedure. Specific kind of mutation 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 gene mutation and the HEX (or VIC) signal indicates internal control.

Kit Contents

The kit contains DNA polymerase, positive control, and reaction reagents are pre-loaded in 8-tube strips (Table 1); each PCR strip contains the same B-raf mutation detection reagent and internal control, and each PCR reaction tube contains specific primers, fluorescent probes, dNTPs, $MgCl_2$, etc. The FAM signal indicates the genetic mutation and the HEX (VIC) signal indicates the internal control.

Table 2. Kit Contents

Content Name	Components	Volume	Quantity
B-raf 8-Tube Strips	Primers, probes, Mg^{2+}, dNTPs	35 µL	3 strips
B-raf Taq Polymerase	Taq DNA polymerase	15 µL	1 tube
B-raf Positive Control	Positive plasmid DNA, wild type DNA	100 µL	1 tube

Note: The contents of different batches cannot be mixed.

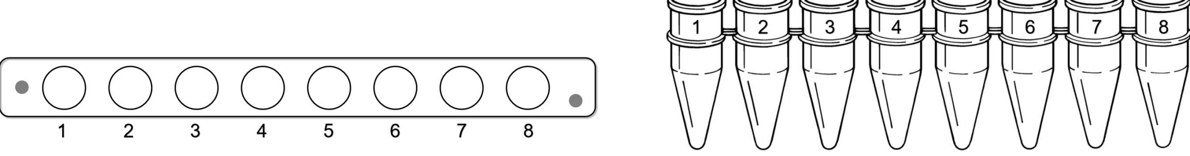

Figure 1. Tube Sequence of 8-Tube Strip

Note: The reaction solutions are pre-loaded in 8-tube strips, as shown in Figure 1.

Additional required Equipment and Materials

1. Commercialized nucleic acid extraction kit
2. Nuclease-Free water (NTC)
3. Aerosol-barrier pipette tips

Transportation, Stability and Storage

1. Storage Condition. Store the kit away from light at -15℃ to -25℃, valid for 12 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

Stratagene Mx3000P™, ABI7500, SLAN-48P/96S, ABI StepOne Plus, etc.

1. For Stratagene Mx3000P™, FAM and HEX channel signal gain multiple is adjusted to 1.
2. 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. 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. Freeze-thaw samples no more than 5 times.

Experimental Procedure

1. Reagent Preparation

   Prepare **B-raf 8-tube Strips** and **B-raf Taq Polymerase** according to samples; briefly centrifuge the strips and Taq polymerase; place them on ice and transfer to the sample processing area; detection of **B-raf Positive Control** (PC) and Negative Control (NTC, Nuclease-Free water) in each reaction/run is recommended.

2. Samples Processing

   (1) Sample preparation: Commercialized kit is recommended to extract genomic DNA. Then dilute sample DNA to 2 ng/μL, the dilution volume is for a minimum of 10 μL, which is so called tested DNA.

   (2) Template preparation: Respectively add 0.4 μL B-raf Taq polymerase to 5 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.

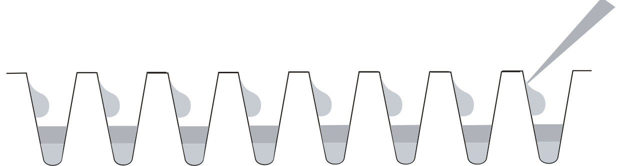

Figure 2. The 8-Tube Strip Sampling Diagram

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; refer to Table 2 for overall arrangement if necessary.

Table 3. Suggested Overall Arrangement

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

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

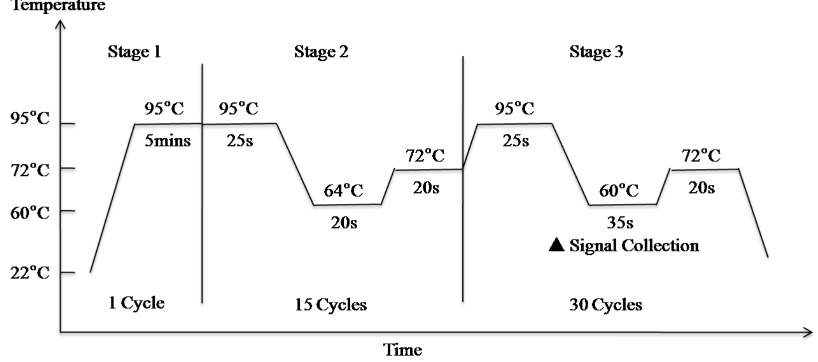

Figure 3. PCR Amplification Procedure

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

Data Analysis

1. The positive judgment value of the B-raf mutation assay in this kit is determined as 28 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) Mutation Result: When the FAM Ct is less than 28, a positive call is returned. When no amplification curve of FAM generates in the well, a negative call or lower than the detection limit of the kit is returned. When $28 \le$ FAM Ct < 30, retest the sample with larger DNA amount; per the retest result, if $28 \le$ FAM Ct < 30, a negative call is returned, if FAM Ct < 28, a positive call is returned.

Interpretation of Results

1. NTC: There should be no amplification curves of FAM in NTC reaction tube, or else, call the result invalid. Occasionally, amplification curve of HEX (VIC) generates in NTC tube, which has no influence on result interpretation.
2. PC: There should be amplification curves of FAM and HEX (VIC), with the value of Ct is less than 20. If the Ct value of FAM or HEX (VIC) in any one tube is greater than 20, the value is invalid and retest is recommended.
3. Internal Control: The HEX (VIC) Ct of every sample reaction tube should be 13-20, which must be qualified before proceeding to further analysis; If the HEX (VIC) Ct is less than 13, that indicates excessive DNA amount, dilute sample DNA for a new detection; If the HEX (VIC) Ct is greater than 20, 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 B-raf gene mutations; 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 qualitative detection of specific V600 mutations of B-raf gene.
5. 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. This kit allows the detection of 1% of specific V600 gene mutations in 10 ng DNA samples.
4. Repeat the test 10 times for the same precision reference material, all of which should be positive, and the coefficient of variation (CV, %) of the Ct value should be less than 10%.
5. There’s no nonspecific product with up to 200 ng wild-type DNA sample.

Warnings and Precautions

1. Please read the instruction carefully in prior to the use of the kit.
2. Avoid repeatedly 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 was manufactured.
LOT	Indicates the manufacturer's batch code so that the batch or lot can be identified.
IVD	Indicates the invitro diagnostic medical device.
	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	Indicate the authorized representative in the European Community
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC.

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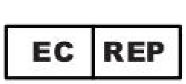

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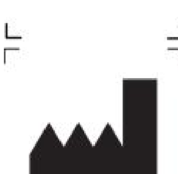

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