PAP-ARMS®

K-ras Gene Mutations Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

Instruction Version: S2.2 Revision Date: July 2023

Product Name

K-ras Gene Mutations Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

6 Tests/Kit, 10 Tests/Kit

Intended Use

This kit uses multiplex fluorescence PCR amplification technology and the DNA extracted from paraffin embedded tissue sections as the detection sample to qualitatively detect 21 hot spot mutation states on codons 12, 13, 59, 61, 117 and 146 of human K-ras gene [1,2] (Attached table 1).

The protein encoded by K-ras gene is a key downstream regulator of EGFR (epidermal growth factor receptor) signal transduction pathway. It participates in the regulation of cell growth and plays an important role in the process of carcinogenesis. K-ras gene mutation will block the upstream EGFR signal pathway and significantly affect the efficacy of EGFR targeted therapeutic drugs. Cetuximab and panimab are monoclonal antibody targeted drugs acting on EGFR, which can inhibit the downstream RAS/RAF/MAPK signal pathway [2-9]. Clinical studies have shown that cancer patients with K-ras gene mutation have no significant effect on the treatment of Cetuximab and Panitumumab [2-10]. Therefore, K-ras gene mutation detection can improve the pertinence of tumor clinical treatment, reduce treatment cost and save valuable treatment time. [2-10]

Technological Principles

The kit uses the sequences of 21 mutation sites of K-ras 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 and external control are conserved sequences on the human genome, with a length of 100 bp. In product analysis, fluorescence labeled probe real-time tracking analysis technology is used to automate the detection method. Fluorescent probe is a fluorescent labeled oligonucleotide probe. The fluorescent group connected to the 5' end of the probe is called the reporter group, and the quencher whose absorption spectrum coincides with the emission spectrum of the reporter group is labeled at the3′ end of the probe is called the quencher group. When the probe is complete, due to the fluorescent group is close to the quenching group, the excited fluorescence is absorbed by the quenching group through resonance energy transfer, which shows that the fluorescence is quenched; In the process of gene amplification, during the extension reaction of the amplified product, the5' exonuclease activity of the polymerase hydrolyzes the fluorescent labeled probe, the fluorescent group is separated from the quenching group and released, showing the fluorescence characteristics, that is, for each amplified DNA chain, a fluorescent molecule is formed, which realizes the complete synchronization between the accumulation of fluorescent signal and the formation of PCR product. The kit realizes the detection of mutations in sample DNA on the real-time PCR platform, achieves high specificity and sensitivity for the detection of rare mutations, and has high selectivity at the same time. When analyzing the results, the gene mutation is indicated by FAM signal, the external control is indicated by FAM signal, and the internal control is indicated by HEX (or VIC) signal.

Kit Contents

The kit adopts the pre-load design of 12-tube strips, and each 12-tube strip detects one sample. There are internal control reagents and 21 kinds of mutation detection for K-ras gene in tube 1-11 of 12-tube strip. The mutation is indicated by FAM signal and the internal control is indicated by HEX/VIC signal; Tube 12, as an external control detection tube for DNA extraction quality, is indicated by FAM signal. Internal control and external control are the quality control of reagents, DNA quality and operation.

Table 1 Kit Contents

Component Name	Contents	6 tests / kit		10 tests / kit	
		Volume	Quantity	Volume	Quantity
K-ras 12-tube reaction strip	Primers, probes, $MgCl_2$, dNTPs	35 μL	8 strips	35 μL	12 strips
K-ras Taq polymerase	Taq DNA polymerase	30 μL	1 tube	50 μL	1 tube

K-ras positive control	Positive plasmid DNA	150 μL	1 tube	150 μL	1 tube

Note: The contents of different batches cannot be mixed.

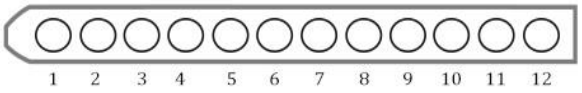

Figure 1 Tube Sequence of 12-tube strip

Note: The reaction solutions are pre-loaded in 12-tube strips, as shown in Figure 1. From left to right are tubes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12.

Materials and Equipment Required but not Provided

1. Microvolume UV-Vis spectrophotometer;
2. Commercialized nucleic acid extraction kit;
3. Nuclease-Free water (NTC);
4. Aerosol-barrier pipette tips;
5. TE buffer solution (pH 8.0).

Transportation, Stability and Storage

1. Storage Condition. Store the kit away from light at -25℃ to -15℃, valid for 9 months. Once opened, the kit is stable at -25℃ to -15℃ until the stated expiration date. Freeze-thaw reagents no more than 5 times.
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 sets as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.

Specimen Material

1. Applicable specimen type: paraffin embedded tissue section, biopsy and surgical resection specimens. After pathological evaluation, it should be ensured to contain at least 30% of tumor cells; The preservation time of paraffin embedded pathological tissue or section samples shall not exceed 2 years.
2. It is recommended to use a commercial kit to extract human genomic DNA, and the obtained DNA 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 re-collected or the sample size shall be expanded before DNA extraction;
3. The extracted DNA is recommended to be tested immediately or stored below -20℃ for no more than 12 months and no more than 5 times of repeated freezing and thawing;

Experimental Procedure

1. Reagent preparation

   Prepare 12-tube strips and K-ras Taq polymerase according to the number of samples; briefly centrifuge the strips and Taq polymerase; place them on ice before transferring to the sample processing area; detection of K-ras Positive Control (PC) and NTC in each reaction/run is recommended.
2. Samples Processing

   (1) Sample preparation: dilute sample DNA to 2 ng/μL with TE buffer solution (pH 8.0). The dilution volume is for a minimum of 65 μL, which is so called tested DNA;

(2) Template preparation: respectively pipet 3.25 μL K-ras Taq polymerase to 65 μL of the diluted DNA sample, PC, and NTC, vortex slightly to mix, then pulse centrifuge;

(3) Gently remove the cap of 12-tube strip, sequentially pipet 5 μL of the templates into tubes of each strip, cover the cap carefully.

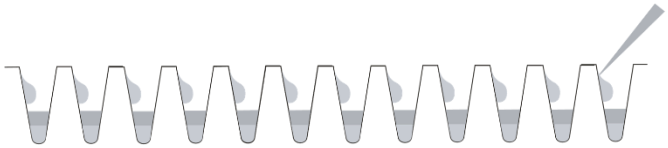

Figure 2 The 12-Tube Strip Sampling Diagram

3. Amplification

(1) Centrifuge the 12-tube strips for 10 seconds to collect templates;

(2) Load the 12-tube strips into the real-time PCR instrument; refer to Table 2 for overall arrangement if necessary;

Table 2 Suggested Overall Arrangement

Name	Kras-1	Kras-2	Kras-3	Kras-4	Kras-5	Kras-6	Kras-7	Kras-8	Kras-9	Kras-10	Kras-11	External control
1	Sample1	Sample1	Sample1	Sample1	Sample1	Sample1	Sample1	Sample1	Sample1	Sample1	Sample1	Sample 1
2	Sample2	Sample2	Sample2	Sample2	Sample2	Sample2	Sample2	Sample2	Sample2	Sample2	Sample2	Sample 2
3	Sample3	Sample3	Sample3	Sample3	Sample3	Sample3	Sample3	Sample3	Sample3	Sample3	Sample3	Sample 3
4	Sample4	Sample4	Sample4	Sample4	Sample4	Sample4	Sample4	Sample4	Sample4	Sample4	Sample4	Sample 4
5	Sample5	Sample5	Sample5	Sample5	Sample5	Sample5	Sample5	Sample5	Sample5	Sample5	Sample5	Sample 5
6	Sample6	Sample6	Sample6	Sample6	Sample6	Sample6	Sample6	Sample6	Sample6	Sample6	Sample6	Sample 6
7	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC
8	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC

(3) Set and run the amplification program as shown in Figure 3. Run real-time PCR and save the file;

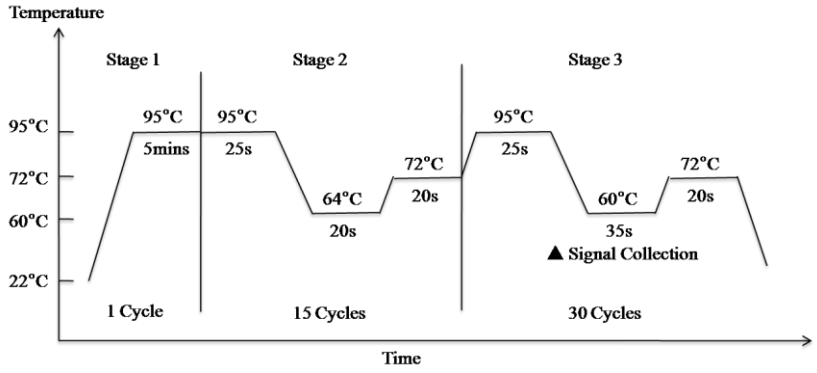

Figure 3 PCR Amplification Procedure

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

Positive Judgment Value

1. 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. Judgment of mutation results (refer to Table 3):

(1) When the FAM mutation Ct value of the sample is greater than or equal to the negative Ct value, the sample is negative or lower than the detection limit of this kit.

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

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

Ct (Mutation): The FAM Ct of tube 1-11 for each sample;

Ct (External): The FAM Ct of tube 12 for each sample.

Table 3 Result Judgment

Mutation Name		Kras-1	Kras-2	Kras-3	Kras-4	Kras-5	Kras-6	Kras-7	Kras-8	Kras-9	Kras-10	Kras-11
positive	critical Ct value	Ct <28	Ct <29	Ct <28	Ct <29	Ct <29	Ct <28	Ct <29	Ct <29	Ct <29	Ct <28	Ct <29
	ΔCt cut-off value	11	11	11	11	11	11	10	13	13	13	13
negative	negative Ct value	Ct≥28	Ct≥29	Ct≥28	Ct≥29	Ct≥29	Ct≥28	Ct≥29	Ct≥29	Ct≥29	Ct≥28	Ct≥29

Interpretation of Results

1. NTC: The FAM signal of tube 1-11 of negative control (NTC) reaction strip shall have no amplification curve; If the FAM signal of any one of tubes 1-11 rises, the experimental result is invalid. If the HEX (or VIC) signal of tube 1-11 or FAM signal of tube 12 rises occasionally, it will not affect the judgment of mutation detection results.
2. PC: The FAM Ct value of positive control (PC) is always less than 20, which can fluctuate with the threshold setting of different instruments.
3. External Control (tube 12): The FAM signal Ct value of the external control reagent reaction tube (tube 12) of each sample to be tested shall be between 13 and 20, and the next analysis shall be carried out after the quality control is qualified; If the Ct value of FAM signal is less than 13, it indicates that the added DNA concentration is too high and should be diluted ,If the FAM signal is negative or the Ct value is greater than 20, it indicates that the added DNA template contains PCR inhibitor or the DNA concentration is too low, which needs to be done after re extracting DNA.
4. Internal Control: The internal control HEX (or VIC) signal of tube 1-11 of the sample to be tested shall have an amplification curve; If the internal control analysis is negative or part of the tube analysis is negative, the result is invalid and needs to be redone.

Limitation of the Kit

1. The test results of this kit are for clinical reference only, and the choice of personalized treatment for patients should be based on their symptoms, signs, medical history and other laboratory tests and treatment response, etc.
2. Negative results could not exclude the existence of K-ras 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 21 mutations sites of K-ras gene.
6. The kit is only applicable with the stated sample types and detection system, including specified instruments, DNA extraction 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 reference materials are 100%.
3. In 10 ng DNA samples, the lower detection limit of mutations detected by Kras-8 and Kras-10 reaction tubes is 5%, and the lower detection limit of other mutation sites is 1%.
4. Repeat the test for the precision reference for 10 times, and the Ct values of FAM and HEX channels are less than 26 (except for HEX channel of external control reaction solution), and the CV value of Ct value is less than 10%.
5. There’s no nonspecific product with up to 50 ng wild-type DNA sample.
6. This kit does not cross-react with E. coli DNA, yeast DNA, Mycobacterium tuberculosis DNA and Streptococcus pneumoniae DNA.

Precautions and Warning

1. Please read this manual carefully before the experiment.
2. Avoid repeatedly freezing and thawing the reagents in the kit.
3. Generally, the amount of DNA should not exceed 10 ng/reaction. In case of poor extraction quality, the amount of DNA can be appropriately increased, especially for paraffin pathological samples fixed with formalin. Due to the cross-linking effect of formalin on

DNA, the DNA in such samples is easy to fragment and degrade. Although the concentration of DNA measured by UV spectrophotometer is high, the amount of effective DNA actually added into the reaction is not enough.

4. The quality of DNA used for detection is very crucial, and the quality control of DNA should be performed after extraction; proceed to sample detection immediately or store sample DNA properly below -20℃.
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 pipet 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 alcoho 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. 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.

Notes

Symbol	Legend
	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 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.
	Indicate the authorized representative in the European Community
	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC.

Literature Reference

1. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC40993, SAKK 60-00 trial. J Clin Oncol, 2010, 28(3):466-474.
2. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol, 2008, 26(10):1626-1634.
3. Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med, 2009, 360(14):1408-1417.

4. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol, 2009, 27(5): 663-671.
5. Baselga J, Rosen N. Determinants of RASistance to anti-epidermal growth factor receptor agents. J Clin Oncol, 2008, 26(10):1582-1584.
6. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predict survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol, 2008, 19(3):508-515.
7. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med, 2008, 359 (17):1757-1765.
8. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol, 2007, 25(22):3230-3237.
9. Lièvre A, Bachet J-B, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol, 2008, 26(3):374-379.
10. Dahabreh IJ, Terasawa T, Castaldi PJ, et al. Systematic review: Anti-epidermal growth factor receptor treatment effect modification by KRAS mutations in advanced colorectal cancer. Ann Intern Med, 2011, 154(1):37-49.
11. Package Insert. Cetuximab (Erbitux®). Branchburg, NJ: ImClone Systems Incorporated; 2012.
12. Package Insert. Vectibix® (Panitumumab). Thousand Oaks, CA: Amgen Inc.; 2012.
13. Consensus Group for KRAS Gene Mutation Detection in Colorectal Cancer. Consensus for KRAS gene mutation detection in colorectal cancer. Chin J Pathol, 2012, 41(09): 635-636.
14. NCCN Clinical Practice Guidelines in Oncology for NSCLC.2015.
15. NCCN Clinical Practice Guidelines in Oncology for CRC.2015.

EC REP

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Attached table 1

KRAS detection site

Tube number	Mutation name	Base change	Cosmic ID
1	G12D	c.35G>A	521
2	G12A	c.35G>C	522
3	G12V	c.35G>T	520
4	G12S	c.34G>A	517
5	G12R	c.34G>C	518
6	G12C	c.34G>T	516
7	G13D	c.38G>A	532
8	G13C	c.37G>T	527
	G13S	c.37G>A	528
	G13R	c.37G>C	529
	K117N	c.351A>C	19940
	K117N	c.351A>T	28519
9	Q61L	c.182A>T	553
	Q61R	c.182A>G	552
	Q61H	c.183A>C	554
	Q61H	c.183A>T	555
10	A59T	c.175G>A	546
	Q61K	c.181C>A	549
11	Al46T	c.436G>A	19404
	A146V	c.437C>T	19900
	A146P	c.436G>C	19905