Jiangsu Mole Bioscience Co., Ltd.
6-7th Floor, G116 Building, No.805, Jiankang Avenue,
Medical New & Hi-tech District,
Taizhou, Jiangsu Province, China
info@molechina.com www.molechina.com

# 7 Kinds of Mutation in *K-Ras* Gene Test Kit

# (Fluorescent Probe-Based Real-Time PCR Assay)

## Instructions for Use

**English VER 1.0**

**REF T021T012B0C2 12 Tests/Box Basic UDI 697089547KRAS00W2**

### PRODUCT NAME

7 Kinds of Mutation In *K-Ras* Gene Test Kit (Fluorescent Probe-Based Real-Time PCR Assay)

### INTENDED USE

This kit is intended for the qualitative detection of 7 common mutations in codons 12 and 13 of the *K-ras* in DNA extracts of tumor tissue samples from patients with lung cancer or colorectal cancer fixed in formalin and paraffin-embedded and can identify the types of mutations.
This kit targeted and specified the 7 common mutations of *K-ras* gene (Table 1), including six base substitution mutations at codon 12 and one base substitution mutation at codon 13. It can be used for individualized molecular diagnosis to assist in individualized therapy for tumor patients.

**Table 1. The 7 Major Somatic Mutations in Codons 12 and 13 of Human *K-ras* Gene**

| Mutation Name | Base Changes | Amino Acid Changes |
|---|---|---|
| 13Asp | GGC>GAC | Glycine to Aspartic acid |
| 12Asp | GGT>GAT | Glycine to Aspartic acid |
| 12Val | GGT>GTT | Glycine to Valine |
| 12Ala | GGT>GCT | Glycine to Alanine |
| 12Arg | GGT>CGT | Glycine to Arginine |
| 12Ser | GGT>AGT | Glycine to Serine |
| 12Cys | GGT>TGT | Glycine to Cystine |

### SUMMARY AND EXPLANATION

*K-ras* oncogene is located on human chromosome 12 and plays an important role in the development of human tumors. *K-ras* acts as a molecular switch: it controls the pathways that regulate cell growth under normal conditions; when abnormalities such as mutations occur, the gene is permanently activated, causing intracellular signal transduction disorders, leading to continuous cell growth and preventing cells apoptosis, and thus leads to cancer. Mutations in the *K-ras* gene occur in the early stages of tumorigenesis, and the *K-ras* genes of the primary and metastatic lesions are highly consistent. It is generally believed that *K-ras* gene status will not change due to treatment. *K-ras* gene is mutated in a variety of tumors, and its incidence is about 40% in colorectal cancer and about 15% in lung cancer. *K-ras* gene mutations mainly occur in codons 12 and 13 of exon 2 (≥90%). These mutations destroy the intrinsic GTPase activity of the *K-ras* protein, thereby making the *K-ras* protein continuously activated.

### TEST PRINCIPLE

This kit uses genomic DNA extracted from tumor tissues of patients with lung cancer or colorectal cancer as a template, and the amplification block mutation system combined with Taqman probe fluorescent quantitative PCR technology is used to detect the *K-ras* gene mutations in the samples. The mutation detection of each sample is carried out in 8 tubes, the reference tube uses the reference primer designed for the wild-type *K-ras* gene, the 7 mutant tubes use the corresponding ARMS primer designed for each mutation, and the probes labeled with 6-FAM and reverse primers used in the 8 tubes are the same. The primers and probe labeled with HEX of the internal control gene are located in the *jak2* gene.

### REAGENTS PROVIDED

| Seq. | Component Name | Ingredients | *No.* (12 Tests) |
|---|---|---|---|
| 1 | PCR mixture 0 (ref) | Buffer, nucleoside triphosphate, magnesium ion, primers and probes, *etc.* | 1 tube (300μL/tube) |
| 2 | PCR mixture 1 (13Asp) | Buffer, nucleoside triphosphate, magnesium ion, primers and probes, *etc.* | 1 tube (300μL/tube) |
| 3 | PCR mixture 2 (12Asp) | Buffer, nucleoside triphosphate, magnesium ion, primers and probes, *etc.* | 1 tube (300μL/tube) |
| 4 | PCR mixture 3 (12Val) | Buffer, nucleoside triphosphate, magnesium ion, primers and probes, *etc.* | 1 tube (300μL/tube) |
| 5 | PCR mixture 4 (12Ala) | Buffer, nucleoside triphosphate, magnesium ion, primers and probes, *etc.* | 1 tube (300μL/tube) |
| 6 | PCR mixture 5 (12Arg) | Buffer, nucleoside triphosphate, magnesium ion, primers and probes, *etc.* | 1 tube (300μL/tube) |
| 7 | PCR mixture 6 (12Ser) | Buffer, nucleoside triphosphate, magnesium ion, primers and probes, *etc.* | 1 tube (300μL/tube) |
| 8 | PCR mixture 7 (12Cys) | Buffer, nucleoside triphosphate, magnesium ion, primers and probes, *etc.* | 1 tube (300μL/tube) |
| 9 | Internal Control | Primers and probes, *etc.* | 1 tube (110μL/tube) |
| 10 | DNA polymerase | DNA polymerase | 1 tube (14μL/tube) |
| 11 | Positive quality control | Synthetic plasmids containing mutated and wild-type *K-ras* gene | 1 tube (70μL/tube) |

## OTHER MATERIALS REQUESTED BUT NOT PROVIDED

The following list includes the materials that are required for use but not included in this Kit:

- Nucleic acid extraction kit.
- Nuclease-free consumables: Filter tips, 1.5mL tubes, PCR-well strips or 96-well plate.
- Experimental equipment: Centrifuge for 1.5mL tubes and PCR-well strips or 96-well plate (if available), Vortex. Real-Time PCR instrument (thermocycler).
- Others: Micropipettes (0.5-20μL, 10-100μL, 20-200μL,100-1000μL), Powder-free disposable gloves, Microplate sealing film**.**

## STORAGE CONDITIONS AND SHELF LIFE

The shelf life of this kit is 12 months when stored in the freezer at - (20±5) ℃. It is suggested to transport the kits in a sealed foam box with dry ice and/or ice packs. The kit is stable within 6 days (including 6 days) during the transportation process (effects were not verified for more than 6 days). Never leave the kit for more than 24 hours at 37℃. Never repeat freeze-thaw more than 6 times for this kit (The effects of repeated freeze-thaw more than 6 times for this kit haven't been verified). After the reagents are opened and mixed, placing them at 2-8℃ for 3 days has no effect on the test results. The effect is not verified for more than 3 days.

## APPLICABLE EQUIPMENT

Applicable to ABI 7500 Real-Time PCR thermocyclers. For other Real-Time PCR thermocyclers, please consult the manufacturer before use.

## ACCEPTABLE SPECIMENS

The samples are paraffin-embedded tumor tissues from patients with lung cancer or colorectal cancer.

## SPECIMENS COLLECTION AND STORAGE

Fresh tissues should be immediately placed in a specimen box or wrapped in a wet cloth, and then transported to the laboratory for segmentation and fixation. Then the samples should be paraffin-embedded in successive steps including fixation, sampling, dehydration, transparency, wax dipping, embedding, sectioning and baking slices. Paraffin-embedded pathological tissue or section samples should be confirmed by experienced pathological doctors to contain tumor cells, and the requested parts should be in the middle of the wax block as much as possible, and non-tumor tissues should be eliminated as much as possible. Pay attention to aseptic operation during sample collection.

A label with a unique identification number is attached to the outside of the sample container. The necessary information should be attached during transportation and storage, such as sample number, date of onset, and date of sample collection. Paraffin-embedded tissues (sections) should be stored at 0-15℃. It has been verified that using paraffin-embedded tissues, which have been stored for 2 years, to extract DNA will still get results, once extracted, the DNA can be stored for 4 months at - (20±5) ℃.

## TEST METHODOLOGY

**1. Nucleic Acid Extraction (Pre-PCR)**

Extract the nucleic acids from the clinical samples and negative control according to the instructions of the nucleic acid extraction kit:

1.1. Clinical samples: Clinical specimen to be tested. The concentration of nucleic acid after extraction should not be less than 5 ng/μL.

1.2. Negative control: normal saline or sterile water supplied by the user.

Note: The proportion of tumor cells in tissue samples should be greater than 80%. Positive quality control does not require extraction.

*We have validated that the kit for nucleic acid extraction from paraffin-embedded tumor tissues is QIAamp DNA FFPE Tissue Kit from Qiagen (Cat. No.56404). If you use nucleic acid extraction kits from other suppliers, please verify first.

**2. Amplification Processes (PCR)**

2.1 Preparation of Amplification Reagent (PCR Room I)

To prepare the PCR reaction mixtures, take the PCR mixture labeled 0-7 tubes, DNA polymerase tube and internal control tube from the kit. Thaw them on ice or at 2-8℃. Shake well and centrifuge all reagent tubes at low speed shortly.

Take 22μL of PCR mixture from tubes labeled 0-7 respectively to each PCR tube/well. Transfer the tubes/wells with PCR mixture, DNA polymerase and internal control to the sample processing area. Refer to the Table below for the sampling layout of a 96-well plate.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PCR mixture 0 | A | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Negative Control | Positive Control |
| PCR mixture 1 | B | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Negative Control | Positive Control |
| PCR mixture 2 | C | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Negative Control | Positive Control |
| PCR mixture 3 | D | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Negative Control | Positive Control |
| PCR mixture 4 | E | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Negative Control | Positive Control |
| PCR mixture 5 | F | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Negative Control | Positive Control |
| PCR mixture 6 | G | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Negative Control | Positive Control |
| PCR mixture 7 | H | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Negative Control | Positive Control |

2.2 Add the Templates (PCR Room II)

Prepare template mixture according to the ratio listed in the table below. After mixing, shake gently, centrifuge at low speed.

| Extracted DNA | Internal Control | DNA Polymerase | Total Volume |
|---|---|---|---|
| 20μL | 8.4μL | 1.0μL | 29.4μL |

Add 3.0 μL template mixture into each PCR well/tube containing PCR mixture. Vortex the sealed plate or tubes to mix well and then centrifuge at 2000-3000rpm for 1min, then transfer to Detection Area for PCR run. Refer to the table in "step 2.1 Preparation of Amplification Reagent (PCR Room I)" for the sampling layout of a 96-well plate.

2.3 Amplification (Detection Area)

Put the reaction tubes/plates into the fluorescent PCR thermocycler and set the cycle parameters as follows:

| Step | Cycles | Temperature (℃) | Time (min.: sec.) |
|---|---|---|---|
| 1 | 1 | 95 | 10:00 |
| 2 | 40 | 95 | 00:05 |
| | | 62* | 00:40 |

Fluorescence signal collection is set as FAM (target gene) and HEX/VIC (internal control). *The signal data collection is set at 62°C. When using the ABI7500, select the 'Quencher' and 'Passive reference' columns as "none". Set the reaction volume per tube/well to 25 μL.

## CUT-OFF VALUE

ROC curve method was used to analyze the results of clinical samples to determine the cut-off values.

| PCR Mixture | Cut-off Value | PCR Mixture | Cut-off Value |
|---|---|---|---|
| PCR mixture 1 (13asp) | 35.6 | PCR mixture 5 (12Arg) | 36.3 |
| PCR mixture 2 (12Asp) | 35.5 | PCR mixture 6 (12Ser) | 35.7 |
| PCR mixture 3 (12Val) | 35.7 | PCR mixture 7 (12Cys) | 36.1 |
| PCR mixture 4 (12Ala) | 36.2 | | |

## EXPLANATION OF THE TEST RESULTS

After the reaction is completed, the instrument automatically saves the results and adjusts the Start, End, and Threshold values of the Baseline after analyzing the image (It is user self-adjustable: Start values can be between 3 and 10. End values can be between 15 and 20). Adjust the amplification curve of the PCR-negative control to straight or below the threshold line.

1. Mutated Ct values refer to Ct values in wells containing PCR mixture 1-7, reference Ct value refers to Ct value in the well-containing PCR mixture 0. A sample may contain multiple mutation types at the same time.
2. The Ct value in HEX/VIC (Internal control) of all reaction wells except negative control should be 20≤Ct≤32, with a typical S-type amplification curve, otherwise, the test for the well is invalid and it should be tested again or the DNA needs to be re-extracted.
3. For positive control, the Ct value in FAM channel of reference wells should be 20≤Ct≤26, with a typical S-type amplification curve. For negative control (self-prepared purified water), the Ct value in all channels of reference and mutation wells should be negative.

The above requirements must be met in the same test at the same time, otherwise, the PCR reaction is considered invalid and should be re-performed. When all of the above requirements are met, the interpretation and judgment of test results are as follows:

| Reaction Well | Ct Value | Interpretation |
|---|---|---|
| PCR mixture 1 (13Asp) | Ct ≤35.6 | 13Asp Mutation positive |
| | Ct > 35.6 | Mutation negative, or below the detection limit |
| PCR mixture 2 (12Asp) | Ct ≤35.5 | 12Asp Mutation positive |
| | Ct > 35.5 | Mutation negative, or below the detection limit |
| PCR mixture 3 (12Val) | Ct ≤35.7 | 12Val Mutation positive |
| | Ct > 35.7 | Mutation negative, or below the detection limit |
| PCR mixture 4 (12Ala) | Ct ≤36.2 | 12Ala Mutation positive |
| | Ct > 36.2 | Mutation negative, or below the detection limit |
| PCR mixture 5 (12Arg) | Ct ≤36.3 | 12Arg Mutation positive |
| | Ct > 36.3 | Mutation negative, or below the detection limit |
| PCR mixture 6 (12Ser) | Ct ≤35.7 | 12Ser Mutation positive |
| | Ct > 35.7 | Mutation negative, or below the detection limit |
| PCR mixture 7 (12Cys) | Ct ≤36.1 | 12Cys Mutation positive |
| | Ct > 36.1 | Mutation negative, or below the detection limit |

If the amount of extracted DNA is low (the average value of multiple measurements is less than 5ng/μL), it is recommended to concentrate or re-extract. Since this kit does not have the function of concentration, whether the concentration could increase the sensitivity has not been verified.

## LIMITATIONS OF THE TEST METHOD

1. The test results of this kit are for clinical reference only, not as the only basis for treatment or other clinical management, the clinical diagnosis and treatment of patients should be comprehensively considered in conjunction with their symptoms/signs, medical history, other laboratory tests and treatment reactions.
2. Negative results cannot completely exclude the presence of target gene mutations, too few tumor cells in the sample, excessive degradation or target gene concentration in the amplification reaction system below the detection limit can also cause negative results.
3. Tumor tissues (cells) may have great heterogeneity, sampling from different parts may result in different test results. Unreasonable sample collection, transportation and processing, as well as improper test operation and experimental environment may lead to false negative or false positive results.
4. The test is limited to the specified sample type and test system (including applicable equipment, nucleic acid extraction reagents, detection methods, *etc.*).

## PRODUCT PERFORMANCE

1. The kit should have a neat appearance, be clearly marked, and have no leakage. After the reagent has melted, it should be clear and transparent without turbidity or precipitation.
2. Coincidence rate of positive reference products: The positive references corresponding to each mutation tube should be detected as corresponding mutation positive.
3. Coincidence rate of negative reference products: 12 negative references should be detected as negative in PCR mixtures 1-7, the positive references should be tested as negative in those wells not corresponding to the specific mutation.
4. LoD: when detecting a 0.2% ratio of mutated DNA in a total genomic DNA with a concentration less than 20ng/μL, the corresponding PCR mixture well should show a positive result.
5. Repeatability: Test two mixed positive references 10 times, PCR mixture 1-7 shows positive results when detected the corresponding mutation and the coefficient of variation of Ct values in each mixture should not be higher than 5.0%.

6. All reaction wells except negative controls should show a positive result of internal control. The internal control of all reference products and positive controls should meet 20≤Ct≤32; the target gene reference wells of positive control products should meet 20≤Ct≤ 26; There is no typical S-type amplification curve in the mutant and reference well of the negative control product (purified water provided by yourself).
7. Inter-batch difference: two mixed positive reference samples were tested for 10 times with three batches of products, PCR mixture 1-7 show positive results when detected the corresponding mutation and the coefficient of variation of Ct values in each mixture should not be higher than 8.0%.
8. The presence of three commonly used chemotherapeutic drugs, 5-fluorouracil (4mg/ml), oxaliplatin (0.8mg/ml), and cytarabine (0.5mg/ml), has no significant effect on the detection results of the kit.

## PRECAUTIONS

1. It is very important to check the quality of all samples. Before extracting genomic DNA from tissue samples, it must be confirmed by an experienced pathologist that the samples contain a sufficient proportion of tumor cells.
2. Avoid repeatedly freezing and thawing the kit. The kit needs to be transported at a low temperature, and it should be frozen when it arrives at the destination.
3. The test is manually operated. Experimental personnel who perform this test should have received professional training in gene amplification or molecular biology diagnostics and be qualified for relevant experimental operations. There should be reasonable biosecurity precautions and protective procedures in the laboratories. The test should only be performed in laboratories that follow safety practices according to the applicable Biosafety Regulations in Microbiological and Biomedical Laboratories.
4. The whole detection process should be carried out in three areas: the first area is for reagent preparation. The second area is for specimen processing and reaction system preparation. The third area is for amplification, fluorescence detection and results analysis. Instruments, equipment and lab coats should be used independently in each area to prevent contamination.
5. In the testing process, should always take care to avoid RNase contamination, wear disposable gloves without fluorescent substances (Frequent replacement is recommended), and use the disposable thin-walled 200μL PCR tube (or 96-well PCR plate with optical film) and pipette tips with filter. Never touch the reaction tube directly with bare hands.
6. The handling of Clinical Specimens should be performed in the biosafety cabinet to ensure the safety of laboratory staff and prevent environmental pollution. Harmful and/or toxic specimens and reagents in the experiment should be properly placed and stored, and in charge by an assigned person. Waste should be disposed of properly in special containers. Lab bench, equipment such as operator's stations, pipettes, centrifuges, and PCR thermocyclers etc., should be regularly wiped and disinfected with 1.0% sodium hypochlorite and/or 70% ethanol. Laboratory room, an ultra-clean bench should be treated with an ultraviolet lamp regularly and after each experiment.
7. Prior to the experiment, reagents should be fully thawed, mixed well, and centrifuged for a few seconds to bring down all the liquid to the bottom of the centrifuge tubes. When preparing the reaction solution, attention should be paid to mixing all liquids on the vortex mixer, not blowing with the pipette to avoid bubbles, and centrifuging the reaction mixture solution for a few seconds. Use the kit before the expiration date and do not combine the reagents with different batch numbers.

**Manufacturing date and expiration date:** view on label

## INDEX OF SYMBOLS

| | | | | | |
|---|---|---|---|---|---|
| | Consult Instructions for Use | Σ | Contain <n> tests | EC REP | Authorized Representative in the European Community |
| IVD | In vitro diagnostic medical device | | Use-by date | -25°C -15°C | Temperature limit -25 to -15°C |
| REF | Catalogue # | LOT | Lot Number | CE | CE conformity marking |
| | Manufacture Date | | Manufacturer | | |

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Medical New& Hi-Tech District,
Taizhou, Jiangsu Province, China
info@molechina.com
www.molechina.com

EC REP **Lotus NL B.V.**
Koningin Julianaplein 10, 1e
Verd, 2595AA, The Hague,
Netherlands.
E-mail: peter@lotusnl.com

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