REF R811PK024

ADPS™

KRAS G12C Mutation Test kit (RUO)

Detection of c.34G>T mutation in exon 2 of the KRAS Gene

Instruction for Use

GENECAST

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Background

KRAS protein is a GTPase and one of the key molecules in the downstream signaling pathway of epidermal growth factor receptor (EGFR). The KRAS mutations are often found on codons 12, 13 and 61 of the exon 2 and 3, and lead to abnormal change of growth signal of the p21-ras protein. These KRAS mutations occur in approximately 30% of lung cancer (NSCLC) and 47% of colorectal cancer (CRC). Especially, KRAS G12C mutation is found in approximately 13% of lung adenocarcinomas, 3% of colorectal carcinoma (CRC), and 1% to 2% of numerous other solid tumors. KRAS G12C is a single point mutation with a glycine-to-cysteine substitution at codon 12. This substitution favors the activated state of KRAS, amplifying signaling pathways that lead to oncogenesis. Existence of KRAS mutations is often related with a prognostic marker to drug response.

The KRAS G12C mutation is found in several cancers, but there is no approved drug that targets this mutation. AMG 510(Amgen) is known as a novel small molecule that specifically inhibits KRAS G12C by inactive to GDP-bound state of KRAS protein. In preclinical study, treatment with AMG 510 led to the regression of KRAS G12C mutant cancer and enhanced the anti-tumour efficacy of chemotherapy and targeted agents. Early detection of KRAS G12Cmutation in cancer patients allows to predict drug response before treatment.

However, our understanding of an individual patient's cancer is often limited by tumor accessibility because of the high risk and invasive nature of current tissue biopsy procedures. "Liquid biopsy", the analysis of circulating tumor DNA (ctDNA), offers a new source of cancer-derived materials that may reflect the status of the disease better and thereby contribute to more personalized treatment. In addition, it is critical to develop more sensitive technologies for detection of mutation.

The ADPS™ KRAS G12C Mutation Test kit (RUO) is a qualitative PCR assay designed to detect KRAS G12C somatic mutations in codon 12. This kit can detect up to 0.01% of the KRAS G12C mutations in DNA extracted from FFPET (Formalin-fixed paraffin-embedded tissue) or plasma and enables qualitative detection.

Intended Use

The ADPS™ KRAS G12C Mutation Test kit (RUO) is a real–time PCR assay for qualitative detection of somatic mutation (c.34G>T) in exon 2 of the KRAS gene. Genomic DNA extracted from whole blood or circulating free DNA extracted from liquid biopsy could be used. The kit is for Research Use Only (RUO), and intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The kit adopts Allele-specific PCR (AS-PCR) and real-time PCR technologies, which comprises specific primers and fluorescent probes to detect *KRAS G12C* mutation in human plasma DNA samples. During the nucleic acid amplification, the targeted mutant DNA is matched with the bases at 3' end of the primer, amplified selectively and efficiently, then the mutant amplicon is detected by fluorescent probes labeled with FAM. While the wild-type DNA cannot be matched with specific primers, there is no amplification occurs.

The kit is composed of ***KRAS G12C* Master Mixture, ADPS™ smart DNA polymerase, *KRAS G12C* Positive Control and nuclease-free water**.

1) The contents in ***KRAS G12C* Master Mixture** formed a mutation detection system and an Internal control system. The mutation detection system includes primers and FAM-labeled probe specific for designated *KRAS G12C* mutation. The internal control system contains the primers and Quasar 670-labeled probe for a KRAS region of genomic DNA without known mutations and polymorphism, to check the presence of inhibitors and monitor the accuracy of

experimental operation.

2) The ***KRAS G12C* Positive Control** contains recombinant plasmid DNA that carries fragments of the *KRAS* gene with G12C mutation.
3) The **ADPS™ smart DNA poymerase** contains ADPS DNA polymerase for PCR amplification

Kit Contents

This kit contains the following materials (see Table 1):

Table 1. Kit Contents (for 24 tests)

Contents	Main Ingredient	Quantity
KRAS G12C Master Mixture	Primers, Probes, dNTPs, Primers/probe for IC*	240 μL/tube ×1
ADPS™ smart DNA polymerase	ADPS™ DNA Polymerase (1 U/μL)	20 μL/tube ×1
KRAS G12C Positive Control	Recombinant plasmid (KRAS G12C) ($3x10^4$ copies/5 μL)	60 μL/tube ×1
Nuclease-free Water	PCR grade water (for no template control, NTC)	500 μL/tube ×1

*IC: Internal control for PCR

The *KRAS G12C* Master Mixture contains all reagents except ADPS™ smart DNA polymerase that is supplied in a separate tube and has to be added before the assay. The detailed detection information is listed in Table 2.

Table 2. Detection Information

Reagent	Fluorescent Signal	
	FAM	Quasar 670**
KRAS G12C Master Mixture	G12C	IC*

*IC: Internal control for PCR

**Alternative dye: Quasar 670 - CY5

Storage and Stability

The kit requires shipment on frozen ice packs. All contents of the kit should be stored immediately upon receipt at

-20±5 ℃ and protected from light.

The shelf-life of the kit is 18 months, and 90 days of opened reagents for the kit. ***KRAS G12C* Master Mixture** and reaction Master Mixtures should be protected from prolonged exposure to light. Reaction Master Mixtures must be stored at 2ºC to 8ºC in the dark. The prepared samples and controls must be added within 1 hour of preparation of the reaction Master Mixtures. Amplification must be started within 1 hour from the time that the processed samples and controls are added to the reaction Master Mixtures.

The recommend maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible PCR instruments: AB 7500 / 7500 FAST, QuantStudio 5, CFX96, CFX384 Real-time system.
2) DNA Extraction kit:

   (Recommended : GeneRead DNA FFPE kit for FFPET sample, QIAamp MinElute ccfDNA kit for plasma sample)

3) Spectrophotometer for measuring DNA concentration.
4) Mini centrifuge with rotor for centrifuge tubes.
5) Mini centrifuge with rotor for PCR tubes.
6) Nuclease-free centrifuge tubes.
7) Nuclease-free PCR tubes and caps.
8) Adjustable pipettors and filtered pipette tips for handling DNA.
9) Tube racks.
10) Disposable powder-free gloves.
11) Sterile, nuclease-free water.

Precautions and Handling Requirements

For research use only.

Precautions

- DO NOT use for *in-vitro* Diagnostic (IVD).
- Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation.
- Please check the compatible real-time PCR instruments prior to use.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Using separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent exogenous DNA contamination to the reagents.
- Please pack the post-amplification tubes with two disposable gloves and discard properly. DO NOT open the post-amplification PCR tubes.
- All disposable materials are for one time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Instrument Setup

- Setup the reaction volume as 20 μL.
- For AB 7500 / 7500 FAST, QuantStudio 5, CFX96, CFX384 Real-time system, please set up as follows: Reporter Dye: FAM, CY5; Quencher Dye: NFQ-MGB; Passive Reference dye: ROX.
- Refer to the real-time PCR instrument operator's manual for detailed instructions.
- We recommend that all PCR instruments in use should be conducted fluorescence calibration once a year.

Assay Procedure

1. DNA Extraction

The specimen material must be human genomic DNA extracted from whole blood samples or circulating DNA extracted from the plasma sample. DNA extraction reagents are not included in the kit. Before DNA extraction, it's essential to use standard pathology methodology to ensure tumor sample quality. Carry out the DNA extraction according to the instructions of DNA extraction kit.

The OD_{260}/OD_{280} value of extracted DNA from whole blood sample or plasma sample should be between 1.7 ~ 2.1 (measured using the spectrophotometer). The storage conditions for extracted DNA from whole blood and plasma used for PCR amplification is shown in Table 3. And the circulating DNA isolated from plasma should be used directly without dilution.

Table 3. Recommended Conditions for Sample and Extracted DNA

	≤ -70°C	**-15°C to -25°C**	**2°C to 8°C**	**15°C to 30°C**
whole blood sample			Up to 3 days	Up to 1 days
Plasma	Up to 12 months		Up to 3 days	
Extracted DNA		Up to 2 freeze thaws over 60 days	Up to 21 days	Up to 7 days

Note:

- *The whole blood sample should be handled and stored properly, and the storage time should preferably be less than 3 days.*
- *The whole blood sample should be derived from EDTA anti-coagulated whole blood samples. The plasma sample should be derived from EDTA anti-coagulated peripheral whole blood. The recommended volume of whole blood is no less than 10 mL,. The PAXgene Blood DNA Tube allows collection of 10mL whole blood into a closed evacuated system.*
- *The extracted DNA should be used immediately, if not, it should be stored at -20±5℃ for no more than 2 months.*
- *Before detection, dilute the extracted DNA with 1×TE buffer (pH 8.0) to designated concentration. We recommend using at least 5 μL DNA for 10 times dilution, to ensure the validity of final concentration.*

2. Mutation Detection

1) Take the ***KRAS G12C*** **Master Mixture**, **KRAS G12C Positive Control**, **Nuclease-free water** and **ADPS™ smart DNA polymerase** out of the kit from the freezer.
2) Thaw the ***KRAS G12C*** **Master Mixture**, **KRAS G12C Positive Control** and **Nuclease-free water** at room temperature. When the reagents are completely thawed, vortex each tubes for 3 sec and centrifuge briefly to collect all liquid at the bottom of the tube.
3) Gently tap the **ADPS™ smart DNA polymerase** and centrifuge briefly prior to use.
4) Prepare sufficient *KRAS G12C* Reaction Mixture containing **ADPS™ smart DNA polymerase**, **KRAS G12C Master Mixture** in separate sterile centrifuge tube respectively according to the ratio in Table 4-1, 4-2. Mix Reaction Master Mix thoroughly by vortexing for 3 sec and centrifuge briefly.

Table 4-1. KRAS G12C Reaction Master Mixture for Mutation Detection

Content	Volume per test (μL)
KRAS G12C Master Mixture	10.0
ADPS™ smart DNA polymerase	0.5
Sample	2.0~9.5
Nulclease-free water	0~7.5
Total volume	20.0

Table 4-2. KRAS G12C Reaction Master Mixture for Positive control

Content	Volume per test (μL)
KRAS G12C Master Mixture	10.0
ADPS™ smart DNA polymerase	0.5
KRAS G12C Positive Control	5.0
Nulclease-free water	4.5
Total volume	20.0

Note:

- *Prepare a positive control experiment at the very end to prevent cross-contamination.*
- *Every PCR run must contain at least one PC (Positive control) and one NTC (No template control).*
- *The prepared mixtures should be used immediately, avoid prolonged storage.*
- *Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.*
- *Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.*

5) Prepare two PCR tubes for each sample: Dispense 10.0 μL of *KRAS G12C* Reaction Mixture to each PCR tube respectively. Then add 5.0 μL of each sample DNA to each sample tube and cap the PCR tubes.
6) Prepare one PCR tube for PC: Dispense 10.0 μL of *KRAS G12C* Reaction Mixture to one PCR tube. Then add PC (5 μL) to sample tube and cap the PCR tube.
7) Add nuclease-free water to all PCR tubes upto 20.0 μL.
8) Briefly centrifuge the PCR strips to collect all liquid at the bottom of each PCR tube.
9) Place the PCR strip tubes into the real-time PCR instrument. A recommended plate layout is shown in Table 5.

Table 5. PCR Plate Layout (Example)

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	Positive Control										
B	Sample 1	Sample 8										
C	Sample 2	Sample 9										
D	Sample 3	Sample 10										
E	Sample 4	Sample 11										
F	Sample 5	Sample 12										
G	Sample 6	Sample 13										
H	Sample 7	Sample 14										

10) Setup the PCR Protocol using the cycling parameters in Table 6.

Table 6. Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	95℃	5 min	-
2	50	95℃	10 sec	-
		60℃	30 sec	**FAM/Quasar670 (CY5)**
		72℃	10 sec	-

11) Run the PCR run immediately.
12) When the PCR operation finished, analyze the data according to the "Results Interpretation" procedures

3. Results Interpretation

Before mutation data analysis, the following items should be checked:

1) Set the threshold to 10% of the maximum fluorescence value of the Positive control (KRAS G12C).

2) For Positive control: FAM Ct value range of the samples should be 24 <Ct < 28.0. If not, the data is INVALID. The sample should be retested.

3) For the Internal control assay for each sample: Ct values of the samples should be 23 <Ct < 26 (Based on copy number of total KRAS gene). If Ct value of IC<23, this indicates that there are too many template in reaction. In this case, it would be better to dilute samples and should be retested. If Ct value of IC>26, this indicates there are insufficient DNA or PCR inhibitors. The sample should be retested with increased or re-extracted DNA.

Analyze the mutation assay for each sample:

1) Record the FAM/Quasar670 Ct values of the samples.

2) Calculate the ΔCt value for each well:

ΔCt value = Sample Ct value (FAM) – Positive control Ct value (FAM)

3) ΔCt Cut-off value: ΔCt < **19.0**

4) Result interpretation for each tube according to the Cut-off ΔCt value in Table 7.

a) If the ΔCt value is< the Cut-off ΔCt value, the sample is determined as positive.
b) If there are no FAM signal and Ct values of sample is 44 < Ct, the sample is determined as negative.
c) If the ΔCt value is ≥ the Cut-off ΔCt value, the sample is determined as negative or under the LoD (limit of Detection) of the kit.

Table 7. Result Determination

KRAS G12C Master Mixture			Determination
	FAM	Quasar670 (Cy5)	
Positive Control Ct value	Ct <24, 28.0<Ct	—	Invalid
Internal Control Ct value	—	Ct <23, 26.0<Ct	Invalid
Sample Ct value	Ct < 23.5		Invalid
	23.5 ≤ Ct < 45.0		Positive
	45 ≤ Ct		Negative
	No signal		Negative
△Ct Cut-off value	△Ct < 19.0		Positive

Performance Characteristics

The performance characteristics of this kit were validated on AB 7500 Fast.

1. Limit of Detection

The Low Limit of Detection (LLoD) of the kit for each mutation is shown in Table 8.

Table 8. LoD for each KRAS G12C mutation

Codon	Mutation	Base Change	COSMIC ID	LoD (copy)	Detection sensitivity (%)
12	G12C	c.34G>T	516	3	0.01

2. Cross-reactivity

Structurally related KRAS protein analog sequences (NRAS and HRAS), KRAS G12D, G12V, G12A, G12S and G12R have been shown not to cross-react with the ADPS™ KRAS G12C Mutation Test kit (RUO) when the potential cross-reactive sequence was added at a genomic copy number equivalent to 99 ng/PCR input to the extracted DNA prior to the amplification. A control condition without plasmid DNA was included. There were no cross-reactivity with each NRAS and KRAS G12 codon mutation plasmids.

3. Interference factor

Triglycerides (37 mM), albumin (60 g/L) and hemoglobin (1.5 g/L) have been shown not to interfere with the ADPS™ KRAS G12C Mutation Test kit (RUO) when the potentially interfering substance was added to healthy-donor K2 EDTA plasma samples containing wild-type and mutant KRAS sequences.

4. Precision

The precision study of the ADPS™ KRAS G12C Mutation Test kit was assessed using diluted KRAS G12C mutation specific plasmids blends with wild-type genomic DNA samples including: 0.02% mutant contents. These samples were tested in duplicate by two operators, using two different reagent lots and two AB 7500 Fast instruments. The Ct values were calculated, the CV values were all within 5%.

Limitations

1) The kit is to be used only by personnel specially trained with PCR techniques.
2) The 0.01 % of detection sensitivity is guaranteed only when input DNA is used with maximum of 30,000 copies.
3) The results should never be used for *in vitro* diagnosis (IVD).
4) The kit has been validated for use with human genomic DNA extracted from whole blood sample and circulating DNA extracted from plasma sample.
5) Reliable results are dependent on proper specimen collection, processing, transport, and storage.
6) The sample containing degraded DNA may affect the ability of the test to detect *KRAS G12C* mutation.
7) This kit can only detect KRAS G12C mutation.
8) Samples with negative result (No mutation detected) may harbor another *KRAS* mutations not detected by this assay.

References

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Symbol Information

Symbol	Title of symbol	Symbol	Title of symbol
	Temperature limit		Caution
	Use-by date		Consult instructions for use
	Batch code		
	Catalogue number		
	Manufacturer (Name&Address)		
	Contains sufficient for <*n*> tests		