KRAS Mutation Real-Time PCR Detection Kit

Screening of KRAS 12 & 13 codon mutations

100 Reactions | -20°C

INTENDED USE

KRAS Mutation Detection Kit is an *in vitro* qualitative kit for the detection of seven somatic mutations located at codon 12 and 13 of KRAS gene in the human genomic DNA extracted from paraffin embedded tissue sections or liquid biopsy samples. The kit is designed to selectively amplify mutant specific sequences in samples that contain a mixture of wild-type and mutated KRAS DNA samples.

SAMPLE TYPE

FFPE Tissue or Liquid Biopsy

BACKGROUND

Cancer is the leading cause of death worldwide and treatment outcomes have been improved dramatically by the advent of mutation-targeted therapies. Kirsten rat sarcoma (KRAS) viral oncogene homolog is the most frequently mutated gene in colorectal (CRC) and non-small cell lung cancer (NSCLC). In their normal state, these cellular proteins in humans play a crucial role in regulating normal tissue signaling processes, including cell proliferation, differentiation, and senescence. KRAS mutations have been reported in codons 12, 13, 59, 61, 117 and 146 and numerous studies have shown that the mutated oncogenes play a vital role in the initiation and progression of cancer. KRAS has been considered a challenging therapeutic target, termed as undruggable over the past four decades. Recent developments on FDA approval for KRAS mutation G12C targeted drugs such as AMG510 (sotorasib) and MRTX849 (adagrasib), has attracted substantial attention.

PERFORMANCE EVALUATION

Performance characteristics has been established by testing the KRAS Mutation Detection Kit with The WHO 1st International Reference Panel for genomic *KRAS* codons 12 and 13 mutations (NIBSC product code 16/250).

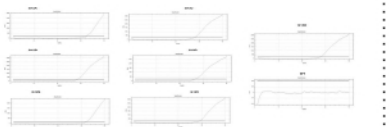

Fig1. Amplification plots indicating detection of NIBSC mutation specific standards using NeoDx KRAS mutation detection Kit (Primer Probe Mix A)

MATERIALS PROVIDED

KIT COMPONENTS	VOLUME
Master Mix	2 X 625 µL
Primer and Probe mix A (Mutations)	125 µL
Primer and Probe mix B (KRAS WT)	125 µL
Nuclease Free Water	2 X 1500 µL
Positive Test Control (PTC) A	40 µL
Positive Test Control (PTC) B	40 µL
Instruction for use	1 No.

PRODUCT DESCRIPTION

KRAS Mutation Detection Kit is a real time PCR *in vitro* diagnostic kit for the detection of 7 mutations in KRAS gene, which includes G12A, G12V, G12C, G12R, G12D, G12S and G13D in genomic DNA extracted from paraffin embedded tissue or liquid biopsy samples along with wild type (wt) KRAS exon 2 as an internal control.

MATERIALS REQUIRED BUT NOT PROVIDED

Consumables
PCR Plates/tubes
PCR plate covers/tube caps

COMPATIBLE INSTRUMENTS

Real-Time PCR, instruments like, BIORAD-CFX96, THERMO -QS5, QIAGEN-ROTOR - GENE Q and other instruments which supports FAM (495 nm - 520 nm).

TARGET (Tube 1)	TARGET (Tube 2)	REPORTER	QUENCHER
KRAS G12 & G13 Mutations	KRAS wt	FAM	BHQ1

*Select the quencher settings as BHQ/None

STORAGE AND HANDLING

- Store all KRAS Mutation Detection kit reagents at -20°C.
- Do not repeatedly freeze-thaw reagents more than five times as it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4°C.
- Kit components are stable through the end of the expiration date indicated on the box when stored at -20°C. Shelf Life - 12 Months from date of manufacturing.

PRECAUTIONS

- It is recommended that this product is used by personnel specially instructed and trained in real-time PCR and in-vitro diagnostics procedures.
- Treat all the specimens as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Keep separate areas for master mix and template preparation and work under biosafety cabinets.
- Use aerosol barrier pipette tips and frequently change the gloves.
- Do not open the reaction tubes/plates post-amplification, to avoid contamination with amplicons.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Do not use kit components that have passed their expiration date.

REACTION MIXTURE - 25 µL (Mutant & WT)*

Reagents	1 Rxn	20 Rxn	50 Rxn	100 Rxn
Master Mix	6.25 µL	125 µL	312.5 µL	625 µL
Primer & Probe mix A/B	1.25 µL	25 µL	62.5 µL	125 µL
Nuclease Free Water	9.5 µL	190 µL	475 µL	950 µL
Total	17 µL	340 µL	850 µL	1700 µL

Add 8 µL of the test DNA per reaction

SAMPLE PREPARATION

DNA should be extracted from FFPE Tissue or Liquid Biopsy aspirates using any approved DNA extraction methods. Store the extracted DNA samples at - 20 °C for further use.

TEST PREPARATION/REACTION SET-UP

- Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 secs and use it immediately.
- Calculate the number of reactions for each experiment including all controls with one excess reaction volume in the reaction cocktail to accommodate pipetting errors. (eg: number of reaction (n) including controls are 10 add 1 extra reaction during the preparation n+1).
- Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples in Master Mix Preparation room.
- Spin down the tubes and dispense 17 µL reaction mix in each tube strips or 96 well plate. Before moving to template adding area, add 8 µL of nuclease free water in NTC wells.
- Carefully add 8 µL of DNA samples kept on ice in the designated wells in template addition room. Add 2 µL of PTC in a separate hood and make up the volume by adding 6 µL of nuclease free water. The assay should be run along with positive controls and negative controls.
- Seal the plate carefully, briefly spin down and use any qRT-PCR instrument which complies with the dyes specified in the kit insert.

THERMAL CYCLIC CONDITIONS

STEP	TEMP °C	TIME	DETECTION	CYCLE
Initial Denaturation	95	2 Min	Off	1
PCR and Detection	95	15 Sec	Off	40
	60	30 Sec	On	

READING TEST RESULTS / DATA ANALYSIS

- NTCs should be negative and should not exhibit fluorescence amplified curves that cross the threshold line.
- If a false positive occurs with one or more of the primer and probe in NTC reactions , it indicates sample contamination.
- In that case, Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.
- Positive control should produce a positive result with an expected Ct value for each target included in the test.
- If expected positive reactivity is not achieved, invalidate the run and repeat the assay with stricter adherence to procedure guidelines.
- After completion of the run, analyze the data as per the instrument manufacturer instructions.
- Analysis should be performed separately for each target using a manual threshold settings.
- In case internal control has not worked for a sample re-do the test with 2 or more dilutions.
- Negative results do not exclude possibility of infection and should not be used as the sole basis for the treatment.

RESULTS INTERPRETATION

KRAS Mutation	KRAS WT	Assay result
Ct < 40	Ct ≤ 40	Mutation Positive
Ct = Undetermined or Ct ≥ 40	Ct ≤ 40	Mutation Negative
Ct = Undetermined or Ct ≥ 40	Ct ≥ 40 Undetermined	Invalid. Re-purify the nucleic acid and repeat the test.

DATA INTERPRETATION

KRAS Mutation (FAM) (Tube 1)	KRAS WT (FAM) (Tube 2)	Results Interpretation
+	+	Indicates presence of KRAS mutation
-	+	Indicates absence of KRAS mutation
-	-	Invalid results repeat the DNA extraction and re-run

WASTE DISPOSAL

- Dispose all the waste/remains of the reagents used in reaction mixture preparation & expired kit components along with bio-waste as per the lab manual/general bio-waste management instruction.
- Dispose the PCR plates with patient samples "sealed" post run to avoid potential infection to the operators and contamination of the lab.

TROUBLESHOOTING

Positive control showed no amplification

Inappropriate storage of reagents

- Store the reagents at recommended temperature for their optimal performance.
- Avoid repetitive freezing and thawing.
- Check the expiry of reagents.

Negative controls are positive

Causes - Cross-contamination

- Follow good laboratory practices to avoid contamination issues.
- Use a new batch of reagents and repeat the experiment.

Abnormal plot and/or low ΔRn values in amplification curve

The baseline was set improperly (some samples have CT values lower than the baseline value)

- Switch from manual to automatic baseline, or move the baseline stop value to a lower CT (2 cycles before the amplification curve for the sample crosses the threshold)

An amplification signal is detected in the early cycles

Dilute the sample to increase the CT value