BRAF V600 Mutation Detection Kit

Qualitative Detection of BRAF V600 mutations

50 Reactions | -20°C

INTENDED USE

BRAF V600 Mutation Detection Kit is an *in-vitro* multiplex, Real-Time PCR assay for the qualitative detection of V600 Mutations and differentiation of V600E mutation in the BRAF gene in human genomic DNA extracted from fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tumor tissue. The Kit differentiates V600E mutation from the other V600 mutations like K/D/Ecomplex/M/R/G.

SAMPLE TYPE

Human genomic DNA extracted from fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tumor tissue

BACKGROUND

High-frequency BRAF mutations have been detected in specific cancers, with melanoma showing approximately 50 to 60% of cases. The presence of BRAF gene mutations is not limited to a particular body part or cell type; it is associated with various cancers, such as Melanoma, Hairy Cell Leukemia, Non-Hodgkin Lymphoma, Thyroid Cancer, Ovarian Cancer, Lung adenocarcinoma, Colorectal cancer, and certain brain cancers like glioblastoma.Around 90% of identified BRAF mutations in human cancer involve a T1799A transversion mutation in exon 15, leading to a V600E amino acid substitution. This V600E mutation accounts for 70 to 90% of BRAF mutant melanoma cases. Additionally, the T1799A alteration might be linked to a second nucleotide mutation (G1798A), resulting in a V600K mutation in an additional ~6% to 29% of patients with a BRAF mutation.The serine/threonine protein kinase BRAF plays a crucial role in the epidermal growth factor receptor (EGFR)-mediated mitogen-activated protein kinase (MAPK) pathway, where activation occurs through the RAS small GTPase.

PRODUCT DESCRIPTION

BRAF V600 Mutation Detection Kit is an in-vitro multiplex Real-Time PCR qualitative assay for the detection of V600 mutations and differentiation of V600E mutations in the BRAF gene in human genomic DNA extracted from fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tumor tissue.

MATERIALS PROVIDED

KIT COMPONENTS	VOLUME
Master Mix	625 µL
Primer and Probe mix	65 µL
Nuclease Free Water	500 µL
Positive Test Control	50 µL
Instruction for use	1 No.

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), Cy5 (649 nm - 670 nm) and HEX (535 nm - 556 nm).

TARGET	REPORTER	QUENCHER
V600 K/D/E complex/M/R/G	FAM	BHQ1
V600 E	Cy5	BHQ2
BRAF Wild	HEX	BHQ2

*Select the quencher settings as BHQ/None

STORAGE AND HANDLING

- Store all BRAF Qualitative 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

Reagents	1 Rxn	20 Rxn	50 Rxn
Master Mix	12.5 µL	250 µL	625 µL
Primer & Probe mix	1.25 µL	25 µL	62.5 µL
Nuclease Free Water	6.25 µL	125 µL	312.5 µL
Total	20 µL	400 µL	1000 µL

Add 5 µL of the test DNA per reaction

SAMPLE PREPARATION

DNA should be extracted from fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tumor tissue aspirates using any approved DNA extraction methods. Store the extracted DNA 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.

- 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 20 µL reaction mix in each tube strips or 96 well plate. Before moving to template adding area, add 5 µL of nuclease free water in NTC wells.
- Carefully add 5 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 3 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
Hold	95	1 Min	Off	1
PCR and Detection	95	5 Sec	Off	40
	50	20 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.

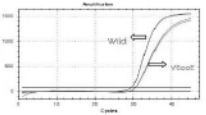
RESULTS INTERPRETATION

For V600 E & K/D/E complex/M/R/G	For BRAF Wild	Assay result
Ct < 40	Ct ≤ 40	Positive
Ct = Undetermined or Ct ≥ 40	Ct ≤ 40	Negative
Ct = Undetermined or Ct ≥ 40	Ct ≥ 40 Undetermined	Invalid. Re-purify the nucleic acid and repeat the test.

DATA INTERPRETATION

V600E	V600 K/D/E complex/M/R/G	Wild	Results Interpretation
+	-	+	V600E detected
-	+	+	V600 K/D/E complex/M/R/G detected
-	-	+	No mutation detected
-	-	-	Invalid results repeat the nucleic acid extraction and re-run

PERFORMANCE CHARACTERISTICS



Performance characteristics has been established by testing the BRAF V600 Mutation Detection kit with Horizon Discovery V600E standard ,50 %

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