BRAF V600E Detection Kit

For Real-Time PCR

REF **PQ3651** Σ **20TESTS** RUO

Wet or dry Ice **Store at: -20°C**

Components

Contents	No. of Tubes	Vol. per Tube (µl)
2x Master Mix Probe	1	200µl
BRAF V600E Detection Primer/Probe Mix*	1	80µl
Control Positive 1% BRAF V600E mutant	1	20µl

* The primer mix tube contains primers/probes for both BRAF V600E (FAM) and endogenous control gene (HEX).

For Use With:

- Applied Biosystems® - 7500/7500 Fast, StepOne/ StepOne Plus
- Qiagen Rotor-Gene® Q
- LightCycler® 480
- QuantStudio™ 5/6
- Applied Biosystems® - 7900HT
- Qiagen Rotor-Gene® 3000, 6000
- Cobas® 4800, Cobas® z480
- Bio-Rad® CFX96

Intended use

BRAF is a serine/threonine kinase that functions within the Ras-Raf-MEK-MAPK pathway. This pathway normally regulates cell proliferation and survival under the control of growth factors and hormones. Mutations in the BRAF gene have been associated with the development of cancer. The most common alteration in the BRAF gene is a mutation called V600E, which alters the valine at position 600 in the protein to a glutamic acid. The V600E mutation causes the BRAF protein to be permanently activated, even in the absence of growth factors. Aberrant BRAF signaling due to the V600E mutation can result in excessive cell proliferation and an adverse resistance to apoptosis. BRAF mutations occur in ~50% of melanoma tumors, ~40% of papillary thyroid tumors, ~30% of ovarian tumors, ~10% of colorectal tumors and ~10% of prostate tumors. The search for drugs that block oncogenic BRAF signaling is an active area of pharmaceutical research and development.

Reagents

Reagent preparation (for all instruments)

The reactions are setup in a total volume of 20µl. Reaction mixes for multiple samples (as well as control samples) should be pre-mixed as a master mix with 5% excess volume to compensate for pipetting losses.

Each 20 µl reaction contains the following components:

Component	Volume (µl)
2x Master Mix Probe	10
BRAF V600E Detection Primer/ Probe Mix	4
DNA sample	4
Molecular Grade Water (RNase/DNase Free)	up to 20

Cycling parameters

Place the plate or strip in the thermal cycler and run the following program:

Temperature	Time	Cycles	Data Collection
95°C	3 min	1	
95°C	10 sec	45	
58°C	40 sec		FAM, HEX

Result Interpretation

For Target Gene BRAF V600E (FAM):

Target gene BRAF V600E Ct is:	Mutation Status
Ct >36	Negative
Ct ≤ 36	Positive

For Endogenous Control Gene (HEX):

Control Gene Ct is:	Control Status
23 ≤ Ct ≤ 28	Sufficient DNA
23 > Ct	Overloaded reaction. May observe non-specific signal. Re-test sample with less DNA to bring HEX within acceptable range.
Ct >28	Low DNA concentration. A rerun with more DNA is recommended.

Signs

Signs	Definitions	Signs	Definitions
RUO	For Research Use Only		Product shipping conditions
	Name and address of the manufacturer of the product	REF	Product technical code

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