

Application Brief

KRAS/BRAF Point Mutation Analysis Panel

Unique All-In-On-Well Assay with High Specificity and Sensitivity

INTRODUCTION

KRAS point mutations are highly specific negative predictors of response to EGFR therapy in patients with advanced Non-Small Cell Lung Cancer (NSCLC) and colorectal cancer (CRC). *BRAF* point mutations have also been associated with lung, colorectal cancers and melanoma. Due to the complexity of these cancers, it becomes increasingly important to be able to test for many point mutations at the same time. By using the ICEPlex® system, researchers can create multiplex assays to test for multiple point mutations in the same well.

PrimeradX has developed a single-well multiplex *KRAS/BRAF* Point Mutation Analysis Panel for detecting and discriminating 17 of the most frequently seen and clinically important mutations in *KRAS/BRAF* genes on the ICEPlex system:

CDS Mutation	Amino Acid
KRAS c.34G>T	G12C
KRAS c.34G>C	G12R
KRAS c.34G>A	G12S
KRAS c.35G>C	G12A
KRAS c.35G>A	G12D
KRAS c.35G>T	G12V
KRAS c.37G>T	G13C
KRAS c.38G>A	G13D
KRAS c.182A>T	Q61L

CDS Mutation	Amino Acid
KRAS c.182A>G	Q61R
KRAS c.183A>T	Q61H-T
KRAS c.183A>C	Q61H-C
KRAS c.436G>A	A146T
BRAF c.1799T>A	V600E
BRAF c.1799_1800TG>AA	V600E
BRAF c.1799_1800GT>AA	V600K
BRAF c.1799_1800TG>AT	V600D

SUMMARY

- Detects 17 *KRAS/BRAF* point mutations in a single well.
- Expedites sample turn-around time to less than 4 hours.
- Detects *KRAS/BRAF* mutations from versatile sample types: FFPE blocks, fresh-frozen tissues, cell lines, serum or plasma.
- Utilizes all-in-one-well assay approach that simplifies lab operation and improves lab economics.
- Requires minimal nucleic acid input and conserves precious samples.

MULTIPLE ANSWERS IN LESS THAN 4 HOURS

METHOD HIGHLIGHTS

- Primers were designed using PrimeraDx's unique strategy that can selectively amplify *KRAS*/*BRAF* point mutations. One of the primers in each primer set was labeled with either FAM or Q-670 dye.
- Nucleic acids were extracted using a commercially available kit either FFPE, tissue, plasma or serum samples.
- Multiplex PCR reactions were subjected to thermocycling in a standard 96-well PCR plate on the ICEPlex system.
- Fluorescently labeled amplicons were then automatically injected, separated and detected in the capillary electrophoresis module of the ICEPlex system.
- Amplification curves for all targets and controls were generated automatically by the built-in ICEPlex software and cycle thresholds (Cts) were calculated.

TYPICAL DATA

The *KRAS*/*BRAF* Point Mutations Analysis Panel of PrimeraDx targeted 17 most frequently seen and clinically important mutations in *KRAS* and *BRAF* genes. The results from the test on ICEPlex system were automatically generated and reported after the end of the run, which took about 3 1/2 hours.

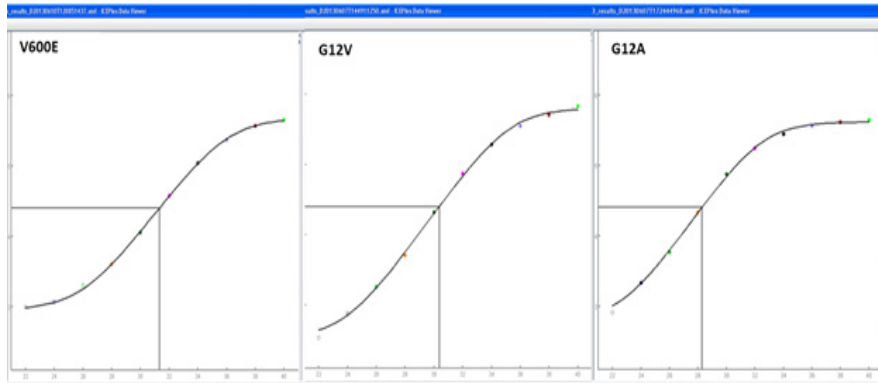


Figure 1. Representative amplification curves for 3 *KRAS*/*BRAF* Mutation Analysis Panel targets on the ICEPlex system.

		G12C	G12R	G12S	G12A	G12D	G12V	G13C	G13D	Q61L	Q61R	Q61H-T	Q61H-C	A146T	V600E-T/A	V600E-TG/AA	V600K	V600D
Sample 1	Ct														31.3			
	Result	No	No	No	No	No	No	No	No	No	No	No	No		Detected	No	No	No
Sample 2	Ct						30.4											
	Result	No	No	No	No	No	Detected	No	No	No	No	No	No		No	No	No	No
Sample 3	Ct				28.3													
	Result	No	No	No	Detected	No	No	No	No	No	No	No	No		No	No	No	No

Table 1. Representative results for three samples on the ICEPlex system.

For a list of publications and to find out more about how PrimeraDx can help your lab, please contact us at 508.618.2300 or visit www.primeradx.com.

The ICEPlex system and the ICEPlex KRAS/BRAF Mutation Assay are for Research Use Only and have not been approved for in vitro diagnostic use by the FDA. The presented information is for demonstration purposes only.