Application Brief

cMET Point Mutation Analysis Panel

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

INTRODUCTION

cMET is a proto-oncogene that encodes the Hepatocyte Growth Factor Receptor (HGFR,) a receptor tyrosine kinase, which plays an essential role in normal cellular function and oncogenesis. In cancer cells, cMET has been implicated in cellular proliferation, cell survival, invasion, cell motility, metastasis and angiogenesis. Recent studies have indicated cMET as a biomarker for various cancers as well as for drug resistance in the case of anti-EGFR therapies. cMET overexpresses or constitutively expresses in cancer cells by several mechanisms. Point mutations in the tyrosine kinase domain can result in constitutive expression/activation of cMET. Here, we report the development of a single-well cMET Point Mutation Analysis Panel, a multiplex PCR assay, which can detect and discriminate 13 cMET point mutations in a single reaction, along with a built-in reference gene on the ICEPlex® system.

CDS Mutation	Amino Acid
c.3172T>C	S1058P
c.3328G>A	V1110I
c.3334C>T	H1112Y
c.3370C>G	H1124D
c.3410G>T	G1137V
c.3446T>C	M1149T
c.3803T>C	M1268T

CDS Mutation	Amino Acid
c.3616G>T	V1206L
c.3637C>G	L1213V
c.3712G>A	V1238I
c.3736G>A	D1246N
c.3743A>G	Y1248C
c.3785A>G	K1262R

SUMMARY

- Detects 13 cMET point mutations from clinical FFPE specimens in a single reaction.
- Requires minimal nucleic acid input and conserves precious samples.
- Simplifies lab operation by applying all-in-one well detection.
- Expedites sample turn-around time to less than 4 hours.

METHOD HIGHLIGHTS

- Primers were designed using PrimeraDx's unique strategy that can selectively amplify cMET point mutations. All primers were analyzed in silico for primer-primer interactions and cross-reactivity. One of the primers in each primer set was labeled with FAM dye.
- Reaction conditions were optimized using proprietary PCR chemistry on the ICEPlex system. Multiplex PCR reactions were carried-out in a standard 96-well PCR plate on the ICEPlex system.
- Fluorescently labeled amplicons for the different cMET point mutations were automatically injected, separated and detected in the capillary electrophoresis module of the ICEPlex system.
- ICEPlex system software plotted the fluorescent signals for different amplicons, generated amplification curves for all targets and controls, and calculated cycle thresholds (Cts).

TYPICAL DATA

Our real-time, single reaction cMET Point Mutation Analysis Panel detected 13 point mutations. The assay had a built-in reference gene, which was used as DNA fragmentation control for FFPE samples. Calibration controls were added and co-amplified in each PCR reaction to mark the sizes of different fluorescently labeled PCR amplicons and to detect PCR inhibition from sample or sample extraction process.

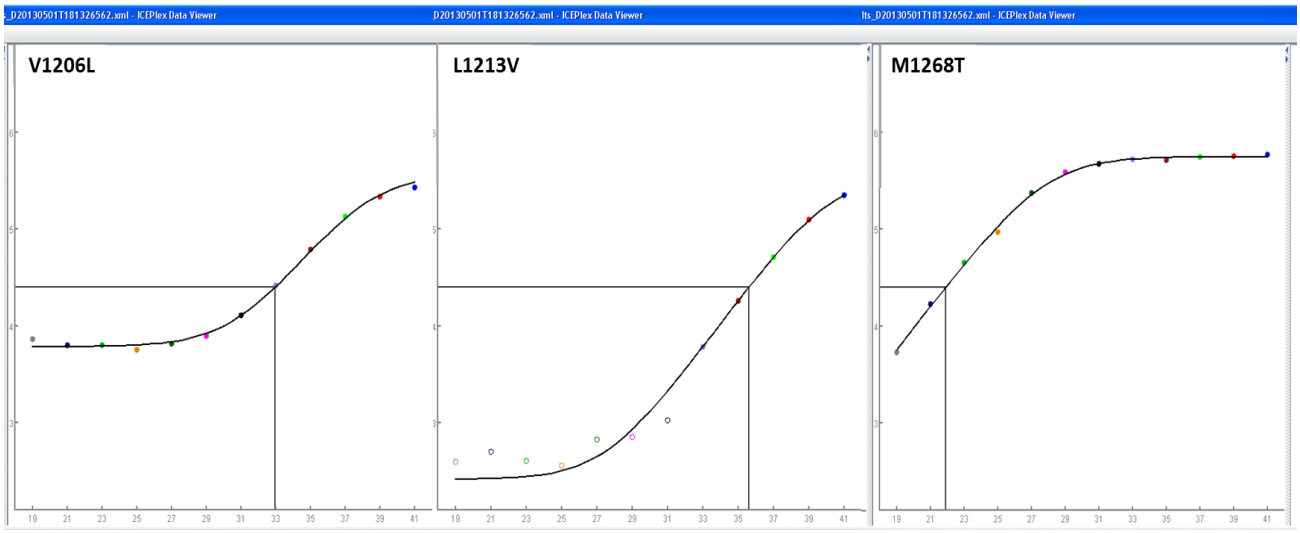

Figure 1 Representative amplification curves for 3 cMET Mutation Panel targets on the ICEPlex system.

		S1058P	V1110I	H1112Y	H1124D	G1137V	M1149T	V1206L	L1213V	V1238I	D1246N	Y1248C	K1262R	M1268T
Sample 1	Ct							32.9						
	Result	No	No	No	No	No	No	Detected	No	No	No	No	No	No
Sample 2	Ct								35.0					
	Result	No	No	No	No	No	No	No	Detected	No	No	No	No	No
Sample 3	Ct													21.9
	Result	No	No	No	No	No	No	No	No	No	No	No	No	Detected

Figure 2. Representative results for three samples.

FOR MORE INFORMATION

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 cMET 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.