

# BRAF Mutation Testing

## TEST DESCRIPTION

Our proprietary assay design, exclusively licensed from New York University, employs allele specific, semi-quantitative PCR for detection of the predominant mutations found at codon 600 of the BRAF oncogene.

The MolecularMD Clinical Laboratory offers sensitive, reproducible and highly specific BRAF mutation testing to aid in patient selection for appropriate therapy and potential monitoring of treatment efficacy.

## CLINICAL UTILITY

BRAF is a member of the Raf family of protein kinases. The BRAF gene codes for a serine/threonine kinase activated within the MAPK signaling pathway involved in cell growth, differentiation and survival. BRAF activating mutations have been identified within a number of solid tumor malignancies including cutaneous melanoma, CRC, NSCLC, and papillary thyroid cancer.<sup>1</sup> Approximately 94% of the mutations reported in BRAF are located in codon 600.<sup>2</sup>

Mutation profiling of tumors aids in the molecular classification of disease subtypes and has guided the development of targeted therapies. Recently, small molecule inhibitors directed against the V600E mutation have demonstrated clinical efficacy in metastatic melanoma patients harboring this mutation.<sup>3</sup> BRAF mutation status in mCRC patients has also been shown to be predictive for response to anti-EGFR therapy.<sup>4</sup>

## CANCER RELEVANCE

- Colorectal cancer
- Thyroid cancer
- Melanoma

## DRUG RELEVANCE

- Anti-EGFR therapies (cetuximab, panitumumab)
- Raf and MEK small molecule inhibitors

## SENSITIVITY

- ~1% mutant allele

## STANDARD TURN AROUND TIME

- 6 days

## RELATED MAPK PATHWAY ASSAYS

- EGFR mutations (PCR assay)
- KRAS codons 12, 13 mutation (PCR assay)
- BRAF exon 15 (sequencing assay)
- NRAS exon 2 codon 61 (sequencing assay)

## EXPERIENCE

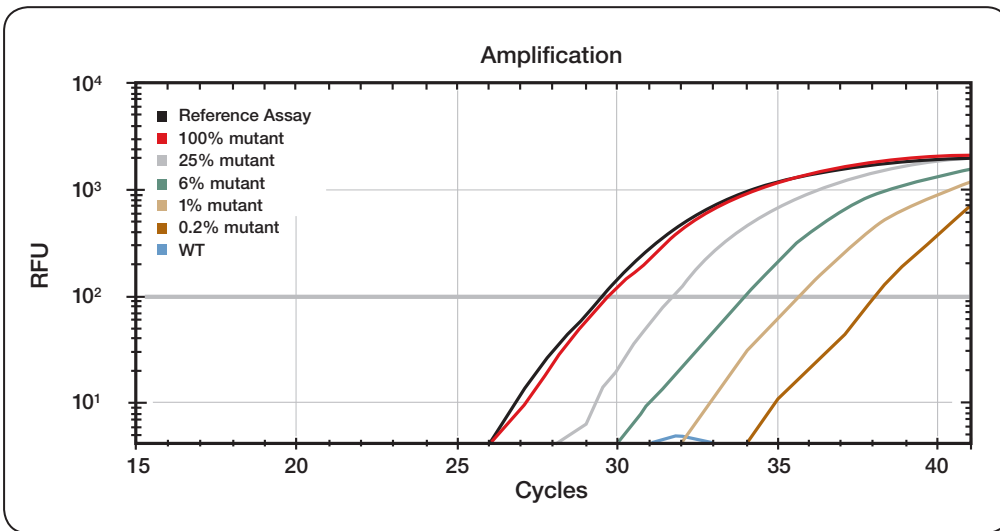
*MolecularMD's centralized CLIA-certified and CAP-accredited molecular diagnostics laboratory has a proven track record in supporting pivotal international clinical research programs. We are a preferred provider of specialty molecular diagnostics services to pharmaceutical and biotech drug developers, offering assays that are rigorously validated to provide rapid and reproducible results that enable prompt clinical decision-making relevant for both solid tumors and hematological malignancies. Our experience and commitment to quality make MolecularMD a leader in reference lab services and an optimal partner for companion diagnostics development*

1. *Cancer Cell* 2003; 4:95-8 2. COSMIC database: <http://www.sanger.ac.uk/genetics/CGP/cosmic/>  
3. *NEJM* 2010; 363:809-819 4. *Lancet Oncol* 2010; 11:753-62

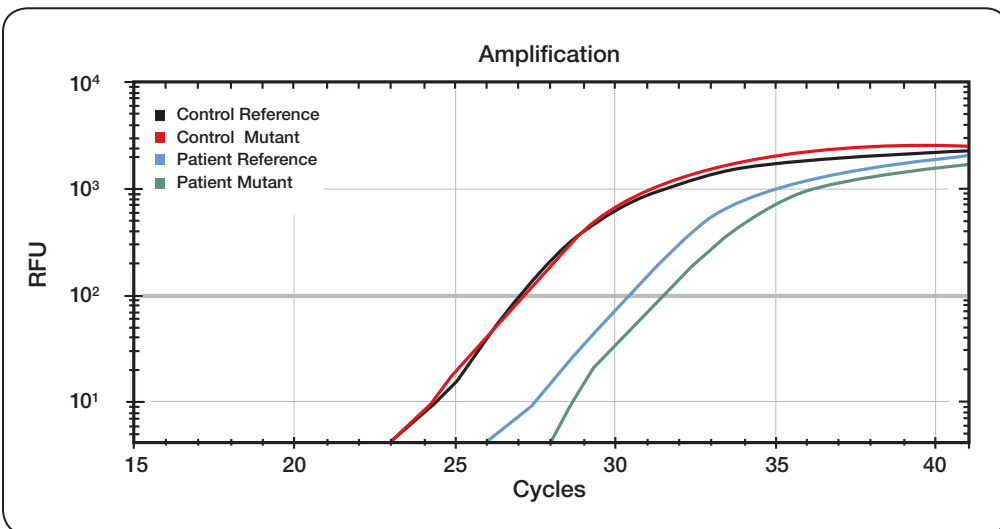
**ASSAY SPECIFICATIONS**

<b>BRAF Mutations Detected</b>	V600E, V600K
<b>Sample Type</b>	Fresh frozen or FFPE tumor tissue (block, sections or core needle biopsy)
<b>Sensitivity</b>	0.2% mutant with intact DNA templates; ~1.0% mutant with FFPE DNA
<b>Sample Requirements</b>	≥1 cm <sup>2</sup> tumor tissue, ≥ 5% tumor cells
<b>Standard Turn Around Time</b>	6 days

**ASSAY PERFORMANCE**



**Figure 1: BRAF Assay Sensitivity.** Serial dilutions of mutant DNA in wild-type DNA were assayed to simulate DNA extracted from tissue of varying tumor content illustrating the dynamic range of the assay. Wild-type DNA (blue trace) template does not amplify with mutant specific primers at >45 cycles illustrating the high specificity of the assay.



**Figure 2: Example of FFPE sample results.** The PCR results for a representative FFPE patient sample are shown with the mutant allele-specific assay (green trace) and the reference assay (blue trace). The delta Ct of approximately 1 indicates a heterozygote. Note: positive control sample traces for the allele specific assay (red trace) and the reference assay (black trace) are also shown.