

# BRAF Mutation Status

## TEST DESCRIPTION

The BRAF oncogene codes for a serine/threonine kinase active in the MAPK cell signaling cascade. Aberrant forms of the BRAF have been identified in 15% of human cancers, including melanoma, colon, and thyroid.<sup>1</sup>

The MolecularMD CLIA/CAP accredited molecular diagnostics laboratory offers sensitive, highly specific BRAF mutation testing to aid clinical research programs and therapy management.

Our proprietary assay design employs Taqman MGB™ probes for allele specific, semi-quantitative PCR (ASQ-PCR) for assessment of the predominant BRAF mutations found at codon 600. A reference control assay utilizes the same reverse primer and probe giving the most accurate measure of the total amount of amplifiable target attainable in the sample (Figure 1).

MolecularMD retains extensive experience in validated analysis of oncogenic mutations in solid tumor tissue samples. Our trusted molecular diagnostic services are supported by world class clinical research leaders and state-of-the-art assay development capabilities.

## CLINICAL UTILITY

BRAF is a member of the Raf family of protein kinases. The BRAF gene codes of a serine/threonine kinase activated within the MAP kinase signaling pathway involved in cellular proliferation. BRAF activating mutations found at codon 600 have been identified within a number of solid tumor malignancies including cutaneous melanoma, NSCLC, and papillary thyroid.<sup>2</sup>

A recent publication presented data showing that CRC patients that harbor mutant BRAF do not respond to anti-EGFR therapies, such as cetuximab and panitumumab.<sup>3</sup> In addition to KRAS mutation analysis, CRC patients should also be screened for BRAF mutations prior to administration of anti-EGFR therapy.

Several BRAF and MAPK targeted therapies are in various stages of clinical development and BRAF molecular diagnostics will continue to evolve as an important test in patient selection and therapy response.

### CANCER RELEVANCE:

- Colorectal (5-22%),
- Papillary Thyroid (30- 80%)
- Melanoma (>60%)

### DRUG RELEVANCE:

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

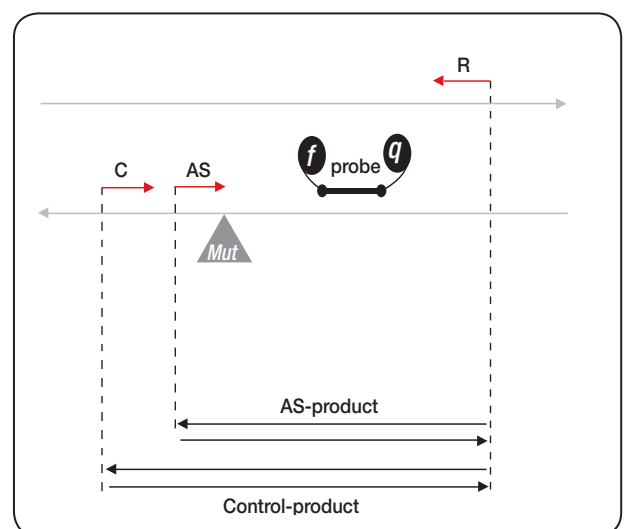
### SENSITIVITY:

- <1% mutant allele

### TURN AROUND TIME:

- 4 to 6 days

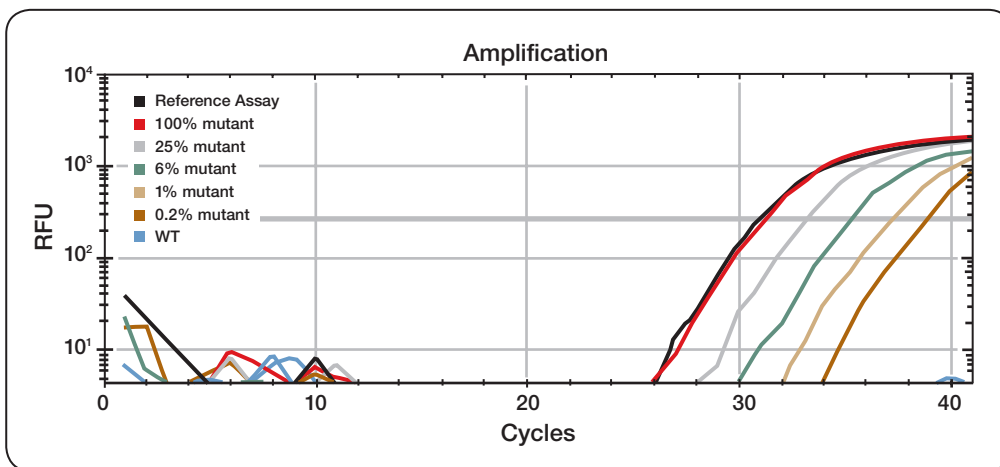
## ASSAY DESIGN



**Figure 1: BRAF assay design using allele specific forward primers to identify the 2 predominant codon 600 mutations.** A reference assay (Control) amplifies the region using the same reverse primer and probe with a non-selective forward primer. Semi-quantitative mutation analysis can be achieved by calculating the delta Ct.

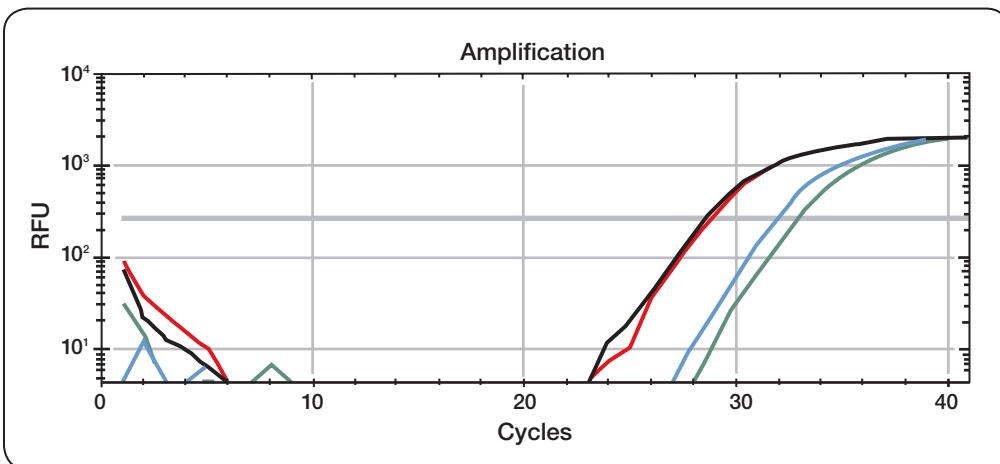
<b>BRAF Mutations Detected</b>	V600E, V600K
<b>Sample Type</b>	Solid tumor from Fresh Frozen (FF) or Formalin Fixed Paraffin embedded (FFPE) tissue (Block or Sections)
<b>Sensitivity</b>	<1% with intact DNA templates (Down to 15 mutant copies in wild-type background). Sufficient sample quality must yield less than 32 cycle thresholds (Ct) for control assay amplification.
<b>Turn Around Time:</b>	4 to 6 Days (Depending on sample quality and sample type)

### ASSAY SENSITIVITY



**Figure 2: BRAF ASQ-PCR Assay Sensitivity.** Serial dilutions of mutant DNA into WT DNA were assayed to simulate DNA extracted from tissue of varying levels of tumor content. Intact DNA template (20ng) is used to demonstrate the sensitivity and linearity of the assay. Wild-type template is undetected, maximizing the dynamic range of the assay.

### PATIENT SAMPLE RESULTS



**Figure 3: Patient Sample Results.** The ASQ-PCR results for a representative FFPE patient sample are shown with the allele specific assay (green trace) and the reference assay (blue trace). The delta Ct of approximately 1 indicates a heterozygote. Note: positive control template traces for both the allele specific assay (red trace) and the reference assay (black trace) are included with each run to validate assay performance.

<sup>1</sup> Davies et al. Mutations of the BRAF gene in human cancer. <sup>2</sup> Tuveson DA, Weber BL, Herlyn M. BRAF as a potential therapeutic target in melanoma and other malignancies. *Cancer Cell*. 4:95–8, 2003.  
<sup>3</sup> Di Nicolantonio et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol*. 26:5705-12, 2008. *Nature*. 417(6892):949-954, 2002.