

KRAS Mutation Status

TEST DESCRIPTION

Detection of KRAS mutations identified with lack of response to Anti-EGFR molecular cancer therapies has developed into a widely adopted test in the management of metastatic colorectal cancer (mCRC). The MolecularMD CLIA/CAP accredited molecular diagnostics laboratory offers precise, sensitive clinical testing to identify the predominant resistant mutant forms of the KRAS oncogene.

Our proprietary assay employs Taqman MGB™ probes for allele specific, semi-quantitative PCR (ASQ-PCR) for assessment of the predominant 7 mutations found at codons 12 and 13. 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 maintains the capacity to process large volumes of KRAS patient tissue samples with rapid turnaround times to support time sensitive international clinical research programs. MolecularMD also offers our dedicated molecular diagnostic services to healthcare providers and their patients who seek the most dependable testing available.

CLINICAL UTILITY

KRAS Codon 12 and 13 mutation analysis has been clinically demonstrated to provide guidance on anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibody therapy. Specifically, somatic mutations identified at codons 12 and 13 have been correlated to a lack of response or survival benefit from EGFR targeted monoclonal therapy, such as panitumumab and cetuximab. Therefore, positive response rates for these therapies are limited to only mCRC patients who harbor wild-type KRAS¹.

The KRAS oncogene encodes the guanosine diphosphate (GDP) and guanosine triphosphate (GTP) binding protein RAS. RAS is down regulated by EGFR and plays a role in the MAPK signaling pathway, a critical pathway involved in cell growth, differentiation, and survival. Percentage of mCRC patients who harbor KRAS mutations ranges between 35% to 45%, depending on the clinical study². Screening of KRAS somatic mutations for mCRC patients is now well integrated into clinical practice.³

CANCER RELEVANCE:

- mCRC
- NSCLC
- Pancreatic

DRUG RELEVANCE:

- Anti-EGFR (cetuximab, panitumumab)
- EGFR small molecular inhibitors (gefitinib, erlotinib)

SENSITIVITY:

- <1% mutant allele

TURN AROUND TIME:

- 4 to 6 days

ASSAY DESIGN

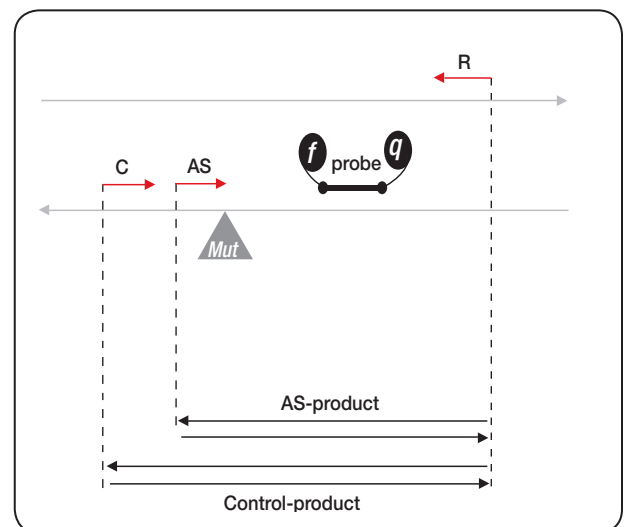


Figure 1: K-RAS assay design using allele specific forward primers to distinguish between the 7 predominant codon 12/13 mutations.

A reference assay (Control) amplifies the region using the same reverse primer and probe with a non-selective forward primer. Semi-quantitative mutation analysis is achieved by calculating the delta Ct.

KRAS Mutations Detected	G12D, G12A, G12V, G12S, G12R, G12C, G13D (Codon 12 and 13)
Sample Type	Solid tumor from Fresh Frozen (FF) or Formalin Fixed Paraffin Embedded (FFPE) tissue (Block or Sections)
Sensitivity	<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.
Turn Around Time:	4 – 6 Days (Depending on sample quality and sample type)

DIAGNOSTIC RESULTS

ASSAY SENSITIVITY

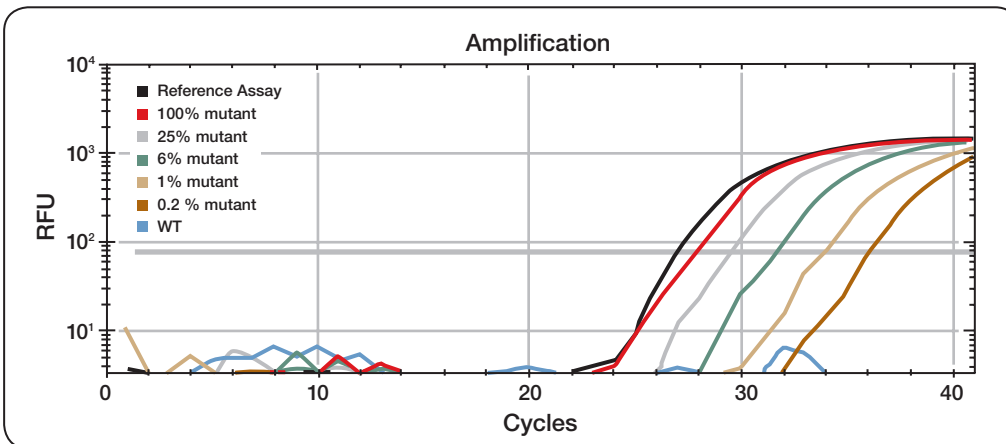


Figure 2: K-RAS Gly12Val ASQ-PCR assay sensitivity. Serial dilutions of mutant DNA into WT DNA were assayed to simulate DNA extracted from tissue of varying levels of tumor content. In-tact DNA template (20ng) is used to demonstrate the sensitivity and linearity of the assay. Wild-type template is undetected maximizing the dynamic range of the assay.

The MolecularMD KRAS testing service provides a powerful platform to evaluate FFPE patient samples with high sensitivity and low background. Each assay includes positive control template and a sample specific reference amplicon to verify reagent and template function.

PATIENT SAMPLE RESULT

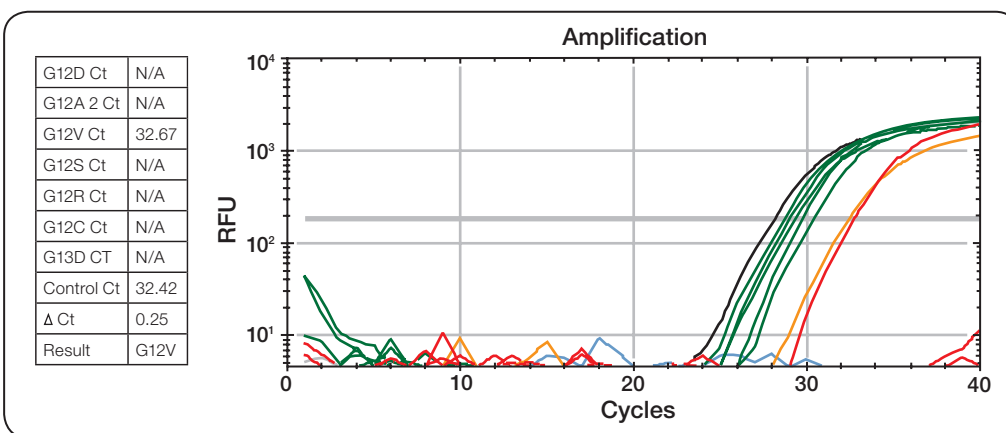


Figure 3. RT-PCR traces for colon FFPE patient sample. Only the ASQ-PCR curves for the mutation G12V, (red trace) and the control assay,(orange trace) are generated. The delta Ct ≤ 1 indicates a high percentage of mutant allele. Note: positive control traces for each ASQ-PCR (green traces) and the control amplicon (black trace) included with each run to validate assay performance.

¹ Amando RG, Wolf M, Peeters M, et al, Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncology*. 2008;26(10):1626-1534. ² Siena S., Sartore-Bianchi A., Bardelli A, et al, Biomarkers Predicting Clinical Outcome of Epidermal Growth Factor Receptor-Targeted Therapy in Metastatic Colorectal Cancer, *J Natl Cancer Inst*, 2009;101:1308-1324. ³ Allegra CJ, Schilsky RL, et al, America Society of Clinical Oncology Provisional Opinion: Testing for KRAS Gene Mutations in Patients with Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy, *J Clin Oncol*, 2009;27:2091-2096.