

KRAS Mutation Testing

TEST DESCRIPTION

Our proprietary assay design employs allele specific, semi-quantitative PCR for detection of the seven predominant mutations found at codons 12 and 13 of the KRAS oncogene.

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

CLINICAL UTILITY

KRAS is a member of the RAS superfamily of GTPases that play an important role in controlling cellular growth, proliferation and differentiation. Mutations in the KRAS gene lead to constitutive activation of the protein and dysregulation of the MAPK pathway. KRAS mutations are frequently detected in many solid tumor malignancies including NSCLC, colorectal cancer and pancreatic cancer. Approximately 90% of the mutations reported in the KRAS gene are located in codons 12 and 13.¹

Mutation profiling of tumors aids in the molecular classification of disease subtypes and has guided the development of targeted therapies. Most significantly, KRAS codon 12/13 mutation status has been demonstrated to predict therapeutic outcome for metastatic CRC patients treated with anti-EGFR therapies such as cetuximab and panitumumab and testing is now well integrated into clinical practice.² Therefore, a sensitive, specific and reproducible method for genotyping KRAS codon 12 and 13 mutations in clinical samples is required to aid in patient selection for appropriate therapy and potential monitoring of treatment efficacy.

CANCER RELEVANCE

- Colorectal cancer
- Non-small cell lung cancer
- Pancreatic cancer

DRUG RELEVANCE

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

SENSITIVITY

- ~1% mutant allele

STANDARD TURN AROUND TIME

- 6 days

RELATED MAPK PATHWAY ASSAYS

- EGFR mutations (PCR assay)
- BRAF codon 600 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. COSMIC database: <http://www.sanger.ac.uk/genetics/CGP/cosmic/> 2. *J Clin Oncol* 2009; 27:2091-96

ASSAY SPECIFICATIONS

KRAS Mutations Detected	G12D, G12A, G12V, G12S, G12R, G12C, G13D
Sample Type	Fresh frozen or FFPE tumor tissue (block, sections, core needle biopsy)
Sample Requirements	≥1 cm ² tumor tissue, ≥ 5% tumor cells
Sensitivity	0.2% mutant with intact DNA templates; ~1% mutant with FFPE DNA
Standard Turn Around Time	6 days

ASSAY PERFORMANCE

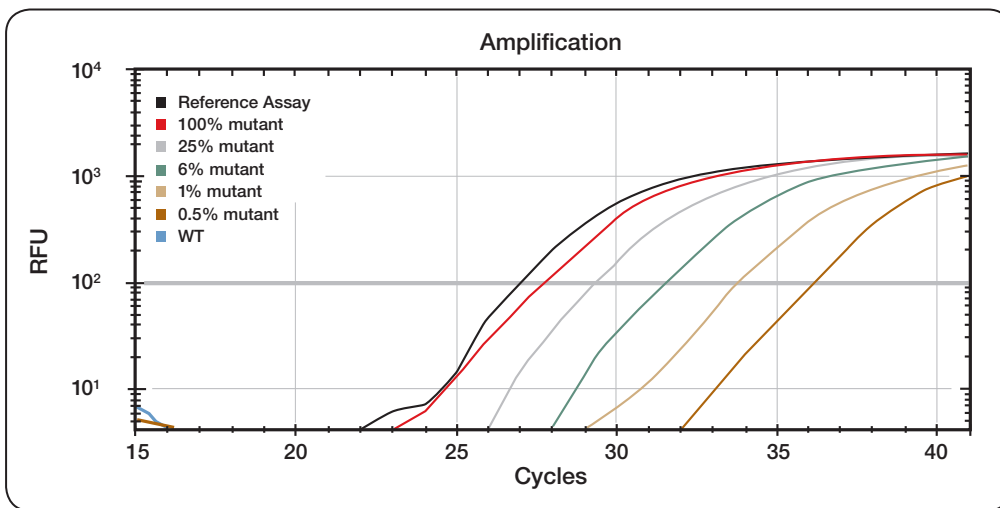


Figure 1: KRAS Assay Sensitivity. Serial dilutions (0.2-100%) of G12V mutant DNA in WT DNA were analyzed to simulate DNA extracted from tissue of varying tumor content. Intact DNA template (20ng) was used to demonstrate the sensitivity and linearity of the assay. Mutant DNA is detectable at the lowest dilution of 0.2% while the 100% WT DNA sample is not amplified at 40 cycles demonstrating the high specificity of the assay.

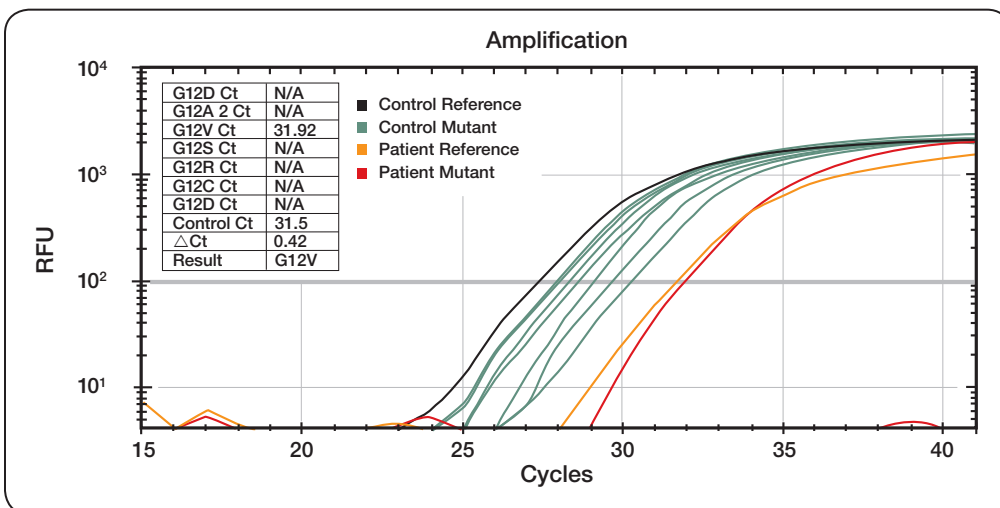


Figure 2: Example of FFPE sample results. Only the PCR amplification curves for the mutation G12V (red trace) and the reference assay (orange trace) are generated. The delta Ct of ≤1 indicates a high percentage of mutant allele. Note: PCR amplification curves are also shown for the Positive Control Sample. Green traces represent the PCR amplification curves for each mutant assay and black trace represents the reference assay. The Positive Control Sample is included with each run to validate assay performance.