

c-KIT Mutation Assays

TEST DESCRIPTION

A panel of bi-directional Sanger sequencing assays has been developed and validated to identify variation in the c-KIT gene using formalin fixed paraffin embedded (FFPE) tissue samples from tumors such as GIST or melanoma.

The panel includes:

1. c-KIT exon 9 - detects the common Ala502_Tyr503dup and any rare mutations in this exon
2. c-KIT exon 11 - detects the wide range of deletions, substitutions, insertions, and duplications present in this exon
3. c-KIT exon 13 - detects the common K642E and N655K mutations associated with melanoma and any rare mutations in this exon
4. c-KIT exon 17 - detects the known D816V/H, Y823D, N822K/H, and D820A/Y/G mutations and any rare mutations in this exon
5. c-KIT exon 18 - detects the known A829P mutation and any rare mutations in this exon

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

CLINICAL UTILITY

The receptor tyrosine kinase c-KIT is a critical regulator of growth, differentiation, migration and proliferation. KIT expression has been reported in a wide variety of cancers. KIT signaling has been most extensively studied in gastrointestinal stromal tumor (GIST) patients.¹ Mutations in c-KIT have been shown to occur in 80 to 85% of GISTs, with many of these described mutations leading to constitutive tyrosine kinase activation. Recent studies have shown that a variety of melanoma sub-types contain c-KIT activating mutations and gene amplifications.² Specific mutations in c-KIT have been shown to be predictive for response to first-line targeted TKI therapy.³ In addition, secondary resistance mutations in c-KIT can be predictive for response to alternative TKI therapies, highlighting the importance of mutation testing in both newly diagnosed and relapsing patients.⁴ Therefore, a sensitive, specific and reproducible method for genotyping c-KIT mutations in clinical samples is required to aid in patient selection for appropriate therapy and potential monitoring of treatment efficacy.

1. *Ann Rev Pathol* 2008; 3:557-586 2. *Clin Can Res* 2008; 14:6821-6828 3. *JCO* 2003; 21:4342-4349 4. *Clin Can Res* 2009; 15:5902-5909

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CANCER RELEVANCE

- GIST
- Melanoma

DRUG RELEVANCE

- c-KIT targeted tyrosine kinase inhibitors

SENSITIVITY

- ~ 20% mutant allele

STANDARD TURN AROUND TIME

- 6 days

RELATED ASSAYS

- PDGFR α exon 12, 14, 18 sequencing assays
- BRAF V600E AS-PCR assay
- BRAF exon 15 sequencing assay
- NRAS exon 2 sequencing assay (codon 61)

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.



c-KIT ASSAY SPECIFICATIONS

Mutations Detected	Ala502_Tyr503dup, K642E, N655K, D816V/H, Y823D, N822K/H, D820A/Y/G and A829P; other rare mutations, deletions, substitutions, insertions, and duplications present in exons 9, 11, 13, 17 and 18.
Sample Type	Fresh frozen or FFPE tumor tissue (block, sections, core needle biopsy)
Sample Requirements	≥1 cm ² tumor tissue, ≥ 50% tumor cells; 1 ug gDNA
Sensitivity	~20% mutant allele
Standard Turn Around Time	6 days

ASSAY PERFORMANCE

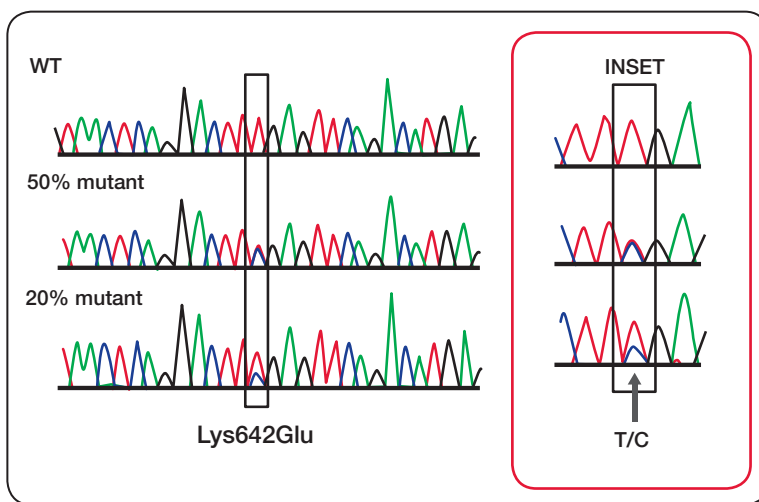


Figure 1: Assay sensitivity. Serial dilutions of K642E mutant DNA in wild-type DNA (20%, 50% and 100% mutant) were assayed to simulate DNA extracted from tissue of varying tumor content. The sequencing chromatogram using the reverse primer is shown for each dilution. The mutated nucleotide is outlined in the black box. A magnified view is shown in the inset box. The mutant peak can be visually detected at 20% in a background of wild-type DNA. Note: blue peak = mutated base (C); red peak = wild-type base (T).

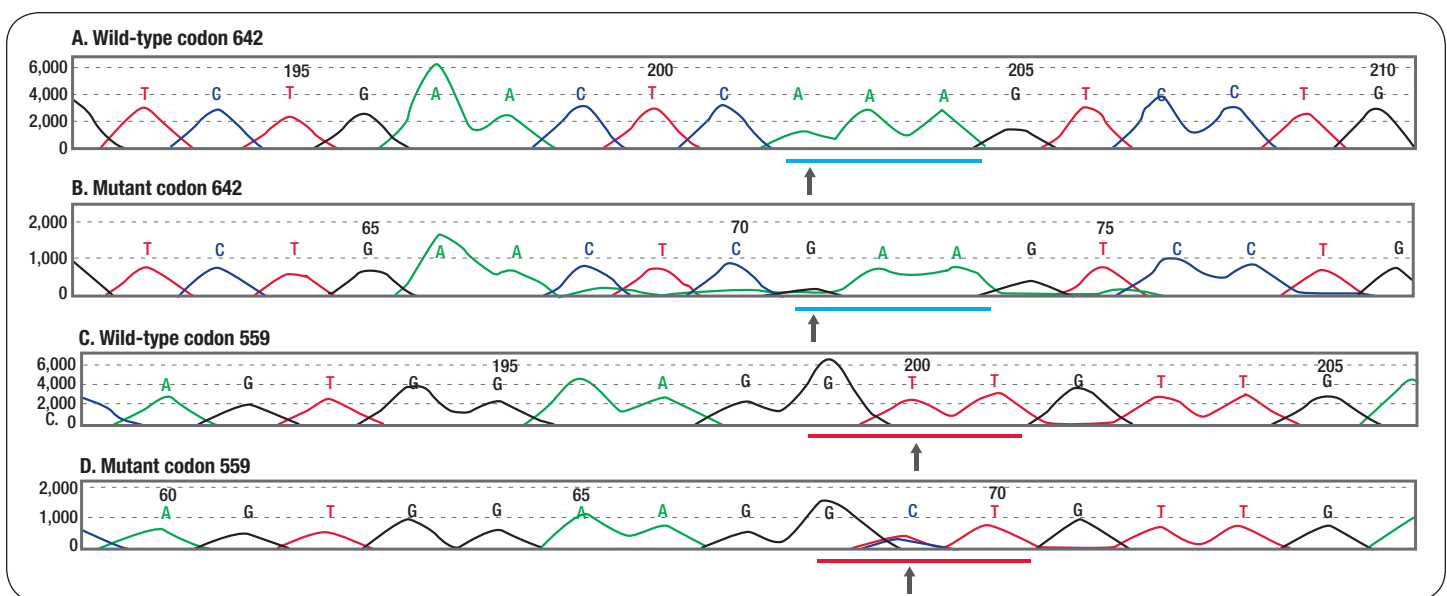


Figure 2: Example of FFPE sample results. (A) Wild-type exon 13 reference sequence. (B) Chromatogram from FFPE tissue sample showing AAA → GAA alteration in codon 642 (K642E) in exon 13. Codon 642 is underscored with a blue bar. The position of the mutated nucleotide is labeled with a black arrow. (C) Wild-type exon 11 reference sequence. (D) Chromatogram from FFPE tissue sample showing GTT → GCT alteration in codon 559 (V559A) in exon 11. Codon 559 is underscored with a red bar. The position of the mutated nucleotide is labeled with a black arrow.