

## Overview

### Useful For

Identifying mutations within the *ALK* gene that predict resistance to ALK-inhibitors

### Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *ALK* gene. See [Targeted Genes and Methodology Details for ALK Mutation Analysis](#) for details regarding the targeted gene regions evaluated by this test.

This test is performed to evaluate for somatic mutations within solid tumor samples. This test **does not assess** for germline alterations within the *ALK* gene.

### Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
SLIRV	Slide Review in MG	No, (Bill Only)	Yes

### Testing Algorithm

When this test is ordered, slide review will always be performed at an additional charge.

### Special Instructions

- [Tissue Requirements for Solid Tumor Next-Generation Sequencing](#)
- [Targeted Genes and Methodology Details for ALK Mutation Analysis](#)

### Highlights

This test evaluates formalin-fixed, paraffin-embedded tumor or cytology slides from patients with solid tumors for gene mutations in the *ALK* gene. Current data suggests that identifying an *ALK* gene mutation may predict resistance to *ALK*-inhibitors.

### Method Name

Sequence Capture Next-Generation Sequencing (NGS)

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

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**Ordering Guidance**

Multiple oncology (cancer) gene panels are available. For more information see [Hematology, Oncology, and Hereditary Test Selection Guide](#).

**Necessary Information**

**A pathology report (final or preliminary), at minimum containing the following information, must accompany specimen for testing to be performed:**

1. Patient name
2. Block number-must be on all blocks, slides, and paperwork (can be handwritten on the paperwork)
3. Tissue collection date
4. Source of the tissue

**Specimen Required**

**This assay requires at least 20% tumor nuclei.**

- Preferred amount of tumor area with sufficient percent tumor nuclei: tissue 216 mm<sup>2</sup>
- Minimum amount of tumor area: tissue 36 mm<sup>2</sup>
- These amounts are cumulative over up to 10 unstained slides and must have adequate percent tumor nuclei.
- Tissue fixation: 10% neutral buffered formalin, not decalcified
- For specimen preparation guidance, see [Tissue Requirement for Solid Tumor Next-Generation Sequencing](#). In this document, the sizes are given as 4 mm x 4 mm x 10 slides as preferred: approximate/equivalent to 144 mm<sup>2</sup> and the minimum as 3 mm x 1 mm x 10 slides: approximate/equivalent to 36 mm<sup>2</sup>.

**Preferred:**

**Specimen Type:** Tissue block

**Collection Instructions:** Submit a formalin-fixed, paraffin-embedded tissue block with acceptable amount of tumor tissue.

**Acceptable:**

**Specimen Type:** Tissue slide

**Slides:** 1 Stained and 10 unstained

**Collection Instructions:** Submit 1 slide stained with hematoxylin and eosin and 10 unstained, nonbaked slides with 5-micron thick sections of the tumor tissue.

**Note:** The total amount of required tumor nuclei can be obtained by scraping up to 10 slides from the same block.

**Additional Information:** Unused unstained slides will not be returned.

**Specimen Type:** Cytology slide (direct smears or ThinPrep)

**Slides:** 1 to 3 Slides

**Collection Instructions:** Submit 1 to 3 slides stained and coverslipped with a preferred total of 5000 nucleated cells, or a minimum of at least 3000 nucleated cells.

**Note:** Glass coverslips are preferred; plastic coverslips are acceptable but will result in longer turnaround times.

**Additional Information:** Cytology slides will not be returned.

**Forms**

If not ordering electronically, complete, print, and send a [Oncology Test Request](#) (T729) with the specimen.

### Specimen Minimum Volume

See Specimen Required

### Reject Due To

Specimens that have been decalcified (all methods) Specimens that have not been formalin-fixed, paraffin-embedded, except for cytology slides Extracted nucleic acid (DNA/RNA)	Reject
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### Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Refrigerated		

## Clinical & Interpretive

### Clinical Information

The *ALK* gene encodes anaplastic lymphoma kinase. Fusions of the *ALK* gene with a variety of 5' (upstream) partner genes upregulate *ALK*'s kinase activity and contribute to tumorigenesis. *ALK* gene fusions have been reported in diverse tumor types. Numerous US Food and Drug Administration approved *ALK*-inhibitors have been developed for the treatment of tumors with *ALK* gene fusions. However, resistance to *ALK* inhibition can occur through the development of *ALK* gene mutations. This test can be used to identify *ALK* resistance mutations to aid in the management of these patients. In addition, *ALK* gain-of-function alterations have been reported in a subset of neuroblastomas.

### Reference Values

An interpretive report will be provided.

### Interpretation

The interpretation of molecular biomarker analysis includes an overview of the results and the associated diagnostic,

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prognostic, and therapeutic implications.

**Cautions**

This test cannot differentiate between somatic and germline alterations. Additional testing may be necessary to clarify the significance of results if there is a potential hereditary risk.

DNA variants of uncertain significance may be identified.

A negative result does not rule out the presence of a variant that may be present but below the limits of detection of this assay. In a specimen with 20% or more tumor content, the analytical sensitivity of this assay for sequence reportable alterations is 5% mutant allele frequency with a minimum coverage of 500X.

Point mutations and small deletion-insertion mutations will be detected in the *ALK* gene only. This test may detect single exon deletions but does not detect multi-exon deletions, duplications, or genomic copy number variants.

Variant allele frequency (VAF) is the percentage of sequencing reads supporting a specific variant divided by the total sequencing reads at that position. In somatic testing, VAF should be interpreted in the context of several factors, including, but not limited to, tumor purity/heterogeneity/copy number status (ploidy, gains/losses, loss of heterozygosity) and sequencing artifact/misalignment.(1,2)

Rare alterations (ie, polymorphisms) may be present that could lead to false-negative or false-positive results.

The presence or absence of a variant may not be predictive of response to therapy in all patients.

Test results should be interpreted in the context of clinical, tumor sampling, histopathological, and other laboratory data. If results obtained do not match other clinical or laboratory findings, contact the laboratory for discussion. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Reliable results are dependent on adequate specimen collection and processing. This test has been validated on cytology slides and formalin-fixed, paraffin-embedded tissues; other types of fixatives are discouraged. Improper treatment of tissues, such as decalcification, may cause polymerase chain reaction failure.

**Supportive Data**

Performance Characteristics:

The limit of detection for calling a somatic variant (single nucleotide variants [SNV] and deletions/insertions [delins, formerly indel]) is 5% variant allele frequency (VAF) and having at least 500x deduplicated coverage.

Verification studies demonstrated concordance between this test and the reference method for detection of SNV and delins is 98.5% (673/683) and 98.4% (122/124) of variants, respectively. Concordance for the detection of delins was 99.0% (100/101) in variants 1 to 10 base pairs (bp) in size, 93.3% (14/15) in variants 11 to 50 bp in size, and 100% (8/8) in variants over 50 bp in size.

To ensure accuracy, this test will be performed on cases that are estimated by a pathologist to have at least 20% tumor cells.

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**Clinical Reference**

1. Strom SP. Current practices and guidelines for clinical next-generation sequencing oncology testing. *Cancer Biol Med*. 2016;13(1):3-11. doi:10.28092/j.issn.2095-3941.2016.0004
2. Spurr L, Li M, Alomran N, et al. Systematic pan-cancer analysis of somatic allele frequency. *Sci Rep*. 2018;8(1):7735. doi:10.1038/s41598-018-25462-0
3. Ou SI, Zhu VW, Nagasaka M: Catalog of 5' Fusion Partners in *ALK*-positive NSCLC Circa 2020. *JTO Clin Res Rep*. 2020 Feb 19;1(1):100015
4. Cooper AJ, Sequist LV, Lin JJ: Third-generation EGFR and ALK inhibitors: mechanisms of resistance and management. *Nat Rev Clin Oncol*. 2022 Aug;19(8):499-514
5. Schneider JL, Lin JJ, Shaw AT: ALK-positive lung cancer: a moving target. *Nat Cancer*. 2023 Mar;4(3):330-343
6. Trigg RM, Turner SD: ALK in Neuroblastoma: Biological and Therapeutic Implications. *Cancers (Basel)*. 2018 Apr 10;10(4):113
7. Brady SW, Liu Y, Ma X, et al: Pan-neuroblastoma analysis reveals age- and signature-associated driver alterations. *Nat Commun*. 2020 Oct 14;11(1):5183

**Performance****Method Description**

Next-generation sequencing is performed to evaluate the presence of a mutation in all coding regions of the *ALK* gene. See [Targeted Genes and Methodology Details for ALK Mutation Analysis](#) for details regarding the targeted gene regions identified by this test. (Unpublished Mayo method)

A pathology review and macro dissection to enrich for tumor cells is performed prior to slide scraping.

**PDF Report**

No

**Day(s) Performed**

Monday through Friday

**Report Available**

12 to 20 days

**Specimen Retention Time**

FFPE tissue block: Unused portions of blocks will be returned 10 to 14 days after testing is complete; FFPE tissue/cytology slides: Unused slides are stored indefinitely; Digital images are obtained and stored for all slides used in testing.

**Performing Laboratory Location**

Rochester

**Fees & Codes**

## Fees

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact [Customer Service](#).

## Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

## CPT Code Information

88381-Microdissection, manual

81479

## LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ALKT	ALK Mutation Analysis, Tumor	In Process

Result ID	Test Result Name	Result LOINC® Value
619695	Result	82939-0
619696	Interpretation	69047-9
619697	Additional Information	48767-8
619698	Specimen	31208-2
619699	Tissue ID	80398-1
619700	Method	48767-8
619701	Disclaimer	62364-5
619702	Released By	18771-6