

## Overview

### Useful For

Identifying *NTRK* mutations that may predict resistance to Trk inhibitors

### Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *NTRK1*, *NTRK2*, and *NTRK3* genes. See [Targeted Genes and Methodology Details for NTRK Genes 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** germline alterations within the genes listed.

### 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 NTRK Genes Mutation Analysis](#)

### Highlights

This test evaluates formalin-fixed, paraffin-embedded tumor or cytology slides from patients with advanced solid tumors for gene mutations in the *NTRK1*, *NTRK2*, and *NTRK3* genes. Current data suggests that identifying an *NTRK* gene mutation may predict resistance to first generation Trk inhibitors.

### Method Name

Sequence Capture Next-Generation Sequencing (NGS)

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

**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(2)
- Minimum amount of tumor area: tissue 36 mm(2)
- 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 Requirements 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(2) and the minimum as 3 mm x 1 mm x 10 slides: approximate/equivalent to 36 mm(2).

**Preferred:** Submit 3, if available, or 2 of the following specimens.

**Acceptable:** Submit **at least one** of the following specimens.

**Specimen Type:** Tissue block

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

**Specimen Type:** Tissue slide

**Slides:** 1 Hematoxylin and eosin-stained and 10 unstained

**Collection Instructions:**

Submit the following slides:

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 total of 5000 nucleated cells (preferred) or at least 3000 nucleated cells (minimum).

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

**Additional Information:** Cytology slides will not be returned. An image of the slides will be stored per regulatory requirements.

### 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

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

### Specimen Stability Information

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

### Clinical & Interpretive

#### Clinical Information

The *NTRK1*, *NTRK2*, and *NTRK3* genes encode the tropomyosin receptor kinases TrkA, TrkB, and TrkC, respectively. Fusions of the *NTRK* genes with a variety of 5' (upstream) partner genes upregulate Trk kinase activity and contribute to tumorigenesis. *NTRK* gene fusions have been reported in diverse tumor types. Numerous US Food and Drug Administration approved pan-Trk inhibitors have been developed for the treatment of tumors with *NTRK* gene fusions. However, resistance to Trk inhibition can occur through the development of *NTRK* gene mutations. This test can be used to identify *NTRK* resistance mutations to aid in the management of these patients. Second generation Trk inhibitors have been developed to overcome resistance to the first-generation inhibitors.

#### 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, prognostic, and therapeutic implications.

#### Cautions

This test cannot differentiate between somatic mutations 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

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this assay. The analytical sensitivity of this assay for sequence reportable alterations is 5% mutant allele frequency with a minimum coverage of 500X in a sample with 20% or more tumor content.

Point mutations and small deletion-insertion mutations (delins) will be detected in the *NTRK1*, *NTRK2*, and *NTRK3* genes only. This test may detect single exon deletions but does not detect multi-exon deletions, duplications, larger-scale genomic copy number variants, copy neutral loss of heterozygosity, or epigenetic modifications such as promoter methylation. Delins of 1000 base pairs or less are detectable with at least 50 or more supporting reads.

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 genetic 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]) 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.

## 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. Published 2018 May 16. doi:10.1038/s41598-018-25462-0
3. Hechtman JF. NTRK insights: best practices for pathologists. *Mod Pathol.* 2022;35(3):298-305

4. O'Haire S, Franchini F, Kang YJ, et al. Systematic review of NTRK 1/2/3 fusion prevalence pan-cancer and across solid tumours. *Sci Rep.* 2023;13(1):4116
5. Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol.* 2018;15(12):731-747
6. Qin H, Patel MR. The Challenge and Opportunity of NTRK Inhibitors in Non-Small Cell Lung Cancer. *Int J Mol Sci.* 2022;23(6):2916
7. Cocco E, Lee JE, Kannan S, et al. TRK xDFG Mutations Trigger a Sensitivity Switch from Type I to II Kinase Inhibitors. *Cancer Discov.* 2021;11(1):126-14
8. Center for Drug Evaluation and Research. Drugs. US Food and Drug Administration. Accessed September 8, 2025. Available at //www.fda.gov/drugs

## Performance

### Method Description

Next-generation sequencing is performed to evaluate the presence of a mutation in all coding regions of the *NTRK1*, *NTRK2*, and *NTRK3* genes.(Unpublished Mayo method)

A pathology review and macro dissection to enrich 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

Tissue blocks: Unused portions of blocks will be returned; Tissue slides: Unused slides are stored for at least 5 years; Extracted DNA: 3 months

### Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

## 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 |
|---------|-------------------------------------|--------------------|
| NTRKM   | NTRK Genes Mutation Analysis, Tumor | 105596-1           |

| Result ID | Test Result Name       | Result LOINC® Value |
|-----------|------------------------|---------------------|
| 619704    | Result                 | 82939-0             |
| 619705    | Interpretation         | 69047-9             |
| 619706    | Additional Information | 48767-8             |
| 619707    | Specimen               | 31208-2             |
| 619708    | Tissue ID              | 80398-1             |
| 619709    | Method                 | 85069-3             |
| 619710    | Disclaimer             | 62364-5             |
| 619711    | Released By            | 18771-6             |