

## Overview

### Useful For

Identifying specific mutations within the *H3-3A*, *H3-3B*, *H3C2*, *H3C3* and *H3C14* genes that assist in tumor diagnosis/classification

### Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *H3-3A* (previously *H3F3A*), *H3-3B* (previously *H3F3B*), *H3C2* (previously *HIST1H3B*), *H3C3* (previously *HIST1H3C*), and *H3C14* (previously *HIST2H3C*) genes. See [Targeted Genes and Methodology Details for Histone Genes, Mutation Analysis](#) for details regarding the targeted gene regions evaluated by this test.

**Note:** This test is performed to evaluate for somatic mutations within solid tumor samples. This test **does not assess** for 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 Histone Genes Mutation Analysis](#)

### 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](#)

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[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 Requirements for Solid Tumor Next-Generation Sequencing](#). In this document, the sizes are given as 4mm x 4mm 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 an [Oncology Test Request](#) (T729) with the specimen.

**Specimen Minimum Volume**

See Specimen Required

## Reject Due To

|  |        |
|--|--------|
| Specimens that have been decalcified (all methods)<br>Specimens that have not been formalin-fixed, paraffin-embedded, except for cytology slides<br>Extracted nucleic acid (DNA/RNA) | Reject |
|--|--------|

## Specimen Stability Information

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

## Clinical & Interpretive

### Clinical Information

*H3-3A* (previously known as *H3F3A*) and *H3-3B* (previously known as *H3F3B*) genes encode H3.3 replication-independent histone proteins. *H3C2* (previously known as *HIST1H3B*) and *H3C3* (previously known as *HIST1H3C*) encode H3.1 replication-dependent histone proteins. *H3C14* (previously known as *HIST2H3C*) encodes H3.2 replication-dependent histone protein. Mutations in *H3-3A* and *H3-3B* genes primarily involve codons K28 (also known as K27) and G35 (also known as G34), whereas mutations in *H3C2*, *H3C3* and *H3C14* involve codon K28 (also known as K27). In central nervous system tumors, *H3-3A*, *H3C2*, *H3C3* and *H3C14* mutations are a diagnostic molecular biomarker for diffuse midline glioma, H3 K27-altered and diffuse hemispheric glioma, H3 G34-mutant. Among bone/soft tissue tumors, *H3-3A* mutations are a hallmark of giant cell tumour of bone and mutations in *H3-3B* and *H3-3A* genes are typical of chondroblastoma.

### 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.

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**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. 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 insertion/deletion mutations will be detected in the *H3-3A*, *H3-3B*, *H3C2*, *H3C3*, and *H3C14* genes 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.

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 and/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 indels]) is 5% variant allele frequency if there is 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 greater than 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. Horbinski C, Nabors LB, Portnow J, et al. NCCN Guidelines Insights: Central Nervous System Cancers, Version 2.2022. *J Natl Compr Canc Netw.* 2023;21(1):12-20

4. WHO Classification of Tumours Editorial Board. Central nervous system tumours. 5th ed. World Health Organization; 2022. WHO Classification of Tumours. Vol 6

5. Sturm D, Witt H, Hovestadt V, et al: Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell.* 2012 Oct 16;22(4):425-437

6. Wu G, Broniscer A, McEachron TA, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet.* 2012 Jan;44(3):251-253

7. Schwartzenuber J, Korshunov A, Liu XY, et al: Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature.* 2012 Jan 29;482(7384):226-231

8. Behjati S, Tarpey PS, Presneau N, et al: Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone. *Nat Genet.* 2013 Dec;45(12):1479-82

## Performance

### Method Description

Next-generation sequencing is performed to evaluate the presence of a mutation in most coding regions of the *H3-3A*, *H3-3B*, *H3C2*, *H3C3*, and *H3C14* genes. See [Targeted Genes and Methodology Details for Histone Genes 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 within 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

81445

### LOINC® Information

| Test ID | Test Order Name                     | Order LOINC® Value |
|---------|-------------------------------------|--------------------|
| HISGT   | Histone Genes Mutation Analysis, Ts | 104062-5           |

| Result ID | Test Result Name       | Result LOINC® Value |
|-----------|------------------------|---------------------|
| 619587    | Result                 | 82939-0             |
| 619588    | Interpretation         | 69047-9             |
| 619589    | Additional Information | 48767-8             |
| 619590    | Specimen               | 31208-2             |
| 619591    | Tissue ID              | 80398-1             |
| 619592    | Method                 | 85069-3             |
| 619593    | Disclaimer             | 62364-5             |
| 619594    | Released By            | 18771-6             |