MAYO CLINIC LABORATORIES

Test Definition: H33T

Histone H3.3 Mutations, Next-Generation Sequencing, Tumor

Overview

Useful For

Identifying specific mutations within the *H3-3A (H3F3A)* and *H3-3B (H3F3B)* 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 (H3F3A)* and *H3-3B (H3F3B)* genes. See Targeted Genes and Methodology Details for Histone H3.3 Mutations for details regarding the targeted gene regions evaluated by this test.

This test is performed to evaluate for somatic mutations within solid tumor samples. It **does not assess** for germline mutations within the genes listed.

Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
SLIRV	Slide Review in MG	No	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 H3.3 Mutations

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Varies

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 4mm x 4mm x 10 slides as preferred: approximate/equivalent to 144 mm(2) and the minimum as 3mm x 1mm x 10 slides: approximate/equivalent to 36 mm(2).

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 slides
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 slides (direct smears or ThinPrep)
Slides: 1 to 3 Slides
Collection Instructions: Submit 1 to 3 slides stained and cover slipped 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	Reject

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

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

H3-3A (formerly *H3F3A*) and *H3-3B* (formerly *H3F3B*) genes encode identical H3.3 core histone proteins that are part of the histone hetero-octamer complex. Mutations in *H3-3A* and *H3-3B* genes primarily involve codons K28 (also known as K27) and G35 (also known as G34) and are increasingly recognized in solid tumors. In central nervous system tumors, *H3-3A* mutations are a diagnostic molecular biomarker for diffuse gliomas and are characteristic of 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.

See Targeted Genes and Methodology Details for Histone H3.3 Mutations for details regarding the targeted gene regions evaluated by this test.

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 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 (H3F3A)* and *H3-3B (H3F3B)* genes only. This test may detect single exon deletions but does not detect multi-exon deletions, duplications, or genomic copy number variants.

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 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 99.7% (699/701) and 96.6% (226/234) of variants, respectively. Concordance for the detection of delins was 98.9% (186/188) in variants 1 to 10 base pairs (bp) in size, 95.8% (23/24) in variants 11 to 50 bp in size, and 88.9% (8/9) in variants 51 to 200 bp in size.

Clinical Reference

1. WHO Classification of Tumours Editorial Board: WHO Classification of Tumours. Vol 6. Central nervous system tumours. 5th ed. World Health Organization; 2021
2.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
3.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 Jan29:44(3):251–253
4. Schwartzentruber 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
5. 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):1479-1482

Performance

Method Description

Next-generation sequencing is performed to evaluate the presence of a mutation in targeted regions of the *H3-3A (H3F3A)* and *H3-3B (H3F3B)* genes. See Targeted Genes and Methodology Details for Histone H3.3 Mutations for details regarding the targeted gene regions evaluated by this test.(Unpublished Mayo method)

A pathology review and macro dissection to enrich for tumor cells are 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 tissue 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 Regional Manager. 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. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

88381-Microdissection, manual
81479

LOINC® Information

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Test Definition: H33T

Histone H3.3 Mutations, Next-Generation Sequencing, Tumor

Test ID	Test Order Name	Order LOINC® Value
H33T	Histone H3.3 Mutations, Tumor	In Process

Result ID	Test Result Name	Result LOINC® Value
617953	Result	82939-0
617954	Interpretation	69047-9
617955	Additional Info	48767-8
617956	Specimen	31208-2
617957	Tissue ID	80398-1
617958	Method	85069-3
617959	Disclaimer	62364-5
617960	Released By	18771-6