MAYO CLINIC LABORATORIES

# Test Definition: CTNBT

B-Catenin Mutation Analysis, Next-Generation Sequencing, Tumor

## Overview

### Useful For

Distinguishing desmoid-type fibromatosis from other soft tissue tumors by assessing gene targets within the beta-catenin (*CTNNB1*) gene

### Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *CTNNB1* gene. See Targeted Genes and Methodology Details for B-Catenin 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. It **does not assess** for germline alterations within the *CTNNB1* gene.

### 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 B-Catenin Mutation Analysis

### Method Name

Sequence Capture and Targeted 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 and Oncology 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 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(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 followings 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**:
1. Hematoxylin and eosin-stained and unstained slides will not be returned.
2. Broken or cracked unstained slides will be rejected. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

**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**:
1. Cytology slides will not be returned. An image of the slides will be stored per regulatory requirements.
2. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

### 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

| | |
|---|---|
| Broken or cracked unstained slides | Reject |
| Broken or cracked stained slides that cannot be repaired | Reject |

### Specimen Stability Information

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

## Clinical & Interpretive

### Clinical Information
Desmoid-type fibromatosis is a locally invasive soft tissue tumor. The histological diagnosis of desmoid-type fibromatosis is challenging. Mutations in exon 3 of the beta-catenin (*CTNNB1*) gene have been identified in 50% to 87% of desmoid-type fibromatosis, including T41A (121 A>G), S45P (133 T>C), and S45F (134 C>T), but not in other soft tissue tumors. Patients harboring beta-catenin mutations may have a higher recurrence rate compared to the patients with wild-type beta-catenin.

This test uses formalin-fixed paraffin-embedded tissue or cytology slides to assess for common somatic mutations in the beta-catenin gene known to be associated with desmoid-type fibromatosis. The results of this test can be useful for supporting a diagnosis of desmoid-type fibromatosis and predicting prognosis.

## 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 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 deletion-insertion mutations (delins) will be detected in the beta-catenin (*CTNNB1*) gene 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 bp 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 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 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.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and will be rejected. Additional material may be requested.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

## 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 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 99.7% (699/701) and 96.2% (226/234), 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. 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. Lazar AJ, Tuvin D, Hajibashi S, et al. Specific mutations in the beta-catenin gene (CTNNB1) correlate with local recurrence in sporadic desmoid tumors. Am J Pathol. 2008;173(5):1518-1527
4. Amary MF, Pauwels P, Meulemans E, et al. Detection of beta-catenin mutations in paraffin-embedded sporadic desmoid-type fibromatosis by mutation-specific restriction enzyme digestion (MSRED): an ancillary diagnostic tool. Am J Surg Pathol. 2007;31(9):1299-1309
5. Domont J, Salas S, Lacroix L, et al. High frequency of beta-catenin heterozygous mutations in extra-abdominal fibromatosis: a potential molecular tool for disease management. Br J Cancer. 2010;102(6):1032-1036

## Performance

### Method Description

Next-generation sequencing is performed to evaluate the presence of a mutation in all coding regions of the *CTNNB1* gene. See Targeted Genes and Methodology Details for B-Catenin Mutation Analysis 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

Tissue blocks: Unused portions of blocks will be returned; Tissue slides: Hematoxylin and eosin-stained and unstained slides will not be returned; Extracted DNA: 3 monthsUnused

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B-Catenin Mutation Analysis, Next-Generation Sequencing, Tumor

### 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 |
|---|---|---|
| CTNBT | B-Catenin Mutations Analysis, Tumor | 103678-9 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 617921 | Result | 82939-0 |
| 617922 | Interpretation | 69047-9 |
| 617923 | Additional Information | 48767-8 |
| 617924 | Specimen | 31208-2 |
| 617925 | Tissue ID | 80398-1 |
| 617926 | Method | 85069-3 |
| 617927 | Disclaimer | 62364-5 |
| 617928 | Released By | 18771-6 |