Test Definition: XL2T

FOXL2 Mutation Analysis, Next-Generation Sequencing, Tumor

Overview

Useful For

Assisting in the clinical diagnosis of adult granulosa cell tumor by assessing gene targets within the *FOXL2* gene

Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *FOXL2* gene.

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

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

Highlights

This test evaluates formalin-fixed, paraffin-embedded tumor or cytology slides to assist in the clinical diagnosis of adult granulosa cell tumor.

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, 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 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: Hematoxylin and eosin-stained and 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 an 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

Granulosa cell tumors (GCT) represent approximately 5% to 10% of all ovarian malignancies and are the most common type of malignant ovarian sex-cord stromal tumors. The majority of GCT (95%) are adult type and 5% are juvenile type. Adult GCT can occur at any age but most commonly occur in perimenopausal women, whereas juvenile GCT arise in the first 3 decades of life. *FOXL2* (forkhead box L2) gene is involved in ovarian development and function. The *FOXL2* gene point mutation c.402C>G in exon 1 (p.C134W) has been reported in the majority of adult GCT (>90%), one molecular subtype of Sertoli-Leydig cell tumor, and rarely also in thecomas. In cases with challenging histopathology, detection of a *FOXL2* mutation can aid in the clinical diagnosis of adult GCT.

This test uses formalin-fixed paraffin-embedded tissue or cytology slides to assess for the common somatic hotspot mutations in the *FOXL2* gene. The results of this test can be useful for supporting a diagnosis of adult GCT.

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 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 (delins) mutations will be detected in the *FOXL2* 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 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 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. 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. Shah SP, Kobel M, Senz J, et al. Mutation of FOXL2 in granulosa-cell tumors of the ovary. N Engl J Med. 2009;360(26):2719-2729
4. Kim MS, Hur SY, Yoo NJ, Lee SH. Mutational analysis of FOXL2 codon 134 in granulosa cell tumour of ovary and other human cancers. J Pathol. 2010;221(2):147-152
5. Schrader KA, Gorbatcheva B, Senz J, et al. The specificity of the FOXL2 c.402C>G somatic mutation: a survey of solid tumors. PLoS One. 2009;4(11):e7988
6. Benayoun BA, Kalfa N, Sultan C, Veitia RA. The forkhead factor FOXL2: a novel tumor suppressor? Biochim Biophys Acta. 2010;1805(1):1-5

7. WHO Classification of Tumours Editorial Board: Female genital tumours. 5th ed. World Health Organization; 2021. WHO Classification of Tumours. Vol 4.

Performance

Method Description

Next-generation sequencing is performed to test for the presence of a mutation in exon 1 of the *FOXL2* gene.(Unpublished Mayo method)

Gene	**GenBank accession number**	**Nucleotide start**	**Nucleotide end**	**Chromosome**	**Exon**
FOXL2	NM_023067	138664432	138665566	Chromosome 3	Exon 1

A pathology review and macro dissection to enrich 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. 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
XL2T	FOXL2 Mutations Analysis, Tumor	95785-2

Result ID	Test Result Name	Result LOINC® Value
617937	Result	82939-0
617938	Interpretation	69047-9
617939	Additional Information	48767-8
617940	Specimen	31208-2
617941	Tissue ID	80398-1
617942	Method	85069-3
617943	Disclaimer	62364-5
617944	Released By	18771-6