



Test Definition: ESR1T

ESR1 Mutation Analysis, Next-Generation Sequencing, Tumor

Overview

Useful For

Assisting in the clinical management of patients with metastatic breast cancer by identifying tumors with evolving resistance to endocrine therapy

Stratifying prognosis of metastatic breast cancer

Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *ESR1* gene. See [Targeted Genes and Methodology Details for ESR1 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 *ESR1* gene.

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, a 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 ESR1 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, 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.

NOTE: Submit tissue from either local recurrence or metastatic disease collected after endocrine therapy has been administered (see Clinical Information for more details).

-Preferred amount of tumor area with sufficient percent tumor nuclei: tissue 216 mm²)

-Minimum amount of tumor area: tissue 36 mm²)

-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 mm²) and the minimum as 3 mm x 1 mm x 10 slides: approximate/equivalent to 36 mm²).

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: 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

The *ESR1* (estrogen receptor 1) gene encodes an estrogen receptor that regulates cell growth through activation of downstream signaling pathways upon binding of estrogen. Tumors demonstrating estrogen receptor expression by immunohistochemistry (ER-positive) are candidates for endocrine therapy, such as selective estrogen receptor modulators (SERM), selective estrogen receptor degraders/downregulators (SERD), and aromatase inhibitors. *ESR1* mutations are rarely observed in untreated breast cancers; however, mutations in the ligand-binding domain of *ESR1* can occur secondarily after exposure to aromatase inhibitors and other endocrine therapies in ER-positive metastatic breast tumors, frequently with multiple different mutations in *ESR1* occurring together. Current data suggests that *ESR1* mutations mediate resistance to endocrine therapy. Studies also suggest that *ESR1* mutations are an independent indicator of poor prognosis.

This test assesses for somatic mutations in *ESR1*, including the ligand-binding domain (exons 4-9 in reference transcript NM_001122740). Breast cancers with mutations in the ligand binding domain of *ESR1* may be responsive to elacestrant (Orserdu), an endocrine therapy in the SERD class of drugs that is clinically approved for postmenopausal women or adult men with ER-positive, *HER2*-negative, *ESR1*-mutated advanced or metastatic breast cancer with disease progression following at least one line of endocrine therapy.

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 deletion-insertion mutations will be detected in the *ESR1* gene only. This test may detect single exon deletions but does not detect multiexon 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 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. Arenedos M, Vicier C, Loi S, et al. Precision medicine for metastatic breast cancer-limitations and solutions. *Nat Rev Clin Oncol.* 2015;12(12):693-704. doi:10.1038/nrclinonc.2015.123
4. Angus L, Beije N, Jager A, et al. ESR1 mutations: Moving towards guiding treatment decision-making in metastatic breast cancer patients. *Cancer Treat Rev.* 2017;52:33-40. doi:10.1016/j.ctrv.2016.11.001
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6. Toy W, Shen Y, Won H, et al. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet.* 2013;45(12):1439-1445. doi:10.1038/ng.2822
7. Robinson DR, Wu YM, Vats P, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat Genet.* 2013;45(12):1446-1451. doi:10.1038/ng.2823
8. Toy W, Weir H, Razavi P, et al. Activating ESR1 mutations differentially affect the efficacy of ER antagonists. *Cancer Discov.* 2017;7(3):277-287. doi:10.1158/2159-8290.CD-15-1523
9. Bidard FC, Kaklamani VG, Neven P et al. Elacestrant (oral selective estrogen receptor degrader) versus standard endocrine therapy for estrogen receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: results from the randomized phase III EMERALD trial. *J Clin Oncol.* 2022;40(28):3246-3256

Performance

Method Description

Next-generation sequencing is performed to evaluate the presence of a mutation in all coding regions of the *ESR1* gene. See [Targeted Genes and Methodology Details for ESR1 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. 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
ESR1T	ESR1 Mutations Analysis, Tumor	102116-1

Result ID	Test Result Name	Result LOINC® Value
617929	Result	82939-0
617930	Interpretation	69047-9
617931	Additional Information	48767-8
617932	Specimen	31208-2
617933	Tissue ID	80398-1
617934	Method	85069-3
617935	Disclaimer	62364-5
617936	Released By	18771-6