

## Overview

### Useful For

Identifying tumors that may respond to targeted therapies by assessing multiple gene targets simultaneously

Identifying mutations that may help determine prognosis for patients with solid tumors

Identifying specific mutations within genes known to be associated with response or resistance to specific cancer therapies

### Genetics Test Information

This extended RAS/RAF panel test uses targeted next-generation sequencing to evaluate for somatic mutations within the *BRAF* (exons 11 and 15), *HRAS* (exons 2 and 3), *NRAS* (exons 2, 3, 4), and *KRAS* (exons 2, 3, 4) genes. This includes, but is not limited to, the testing of somatic mutations in *KRAS* codons 12, 13, 59, 61, 117, 146; *NRAS* codons 12, 13, 59, 61, 146; *HRAS* codons 12, 13, 61; and *BRAF* codons 594, 596, 600. See [Targeted Gene Regions Interrogated by RAS/RAF Gene Panel](#) in Special Instructions for details regarding the targeted gene regions identified by this test.

Of note, this test is performed to evaluate for somatic mutations within 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	Yes

### Testing Algorithm

When this test is ordered, slide review will always be performed at an additional charge.

### Special Instructions

- [Targeted Gene Regions Interrogated by RAS/RAF Gene Panel](#)
- [Tissue Requirements for Solid Tumor Next-Generation Sequencing](#)

### Highlights

This test provides evaluation of *BRAF* (including V600), *HRAS*, *NRAS* (includes codons 12, 13, 59, 61, and 146), and *KRAS* (includes codons 12, 13, 59, 61, 117, and 146) genes for somatic mutations.

### Method Name

Polymerase Chain Reaction (PCR)-Based Next Generation Sequencing

**NY State Available**

Yes

**Specimen****Specimen Type**

Varies

**Ordering Guidance**Multiple oncology (cancer) gene panels are available. For more information see [Oncology Somatic NGS Testing Guide](#).**Necessary Information**

Pathology report (final or preliminary) at minimum containing the following information must accompany specimen in order 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 144 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 Requirement for Solid Tumor Next-Generation Sequencing](#) in Special Instructions. 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 3mm x 1mm x 10 slides: approximate/equivalent to 36mm<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.

**Specimen Type:** Cytology slide (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 1 of the following forms with the specimen:

[-Oncology Test Request \(T729\)](#)

[-Gastroenterology and Hepatology Client Test Request \(T728\)](#)

**Reject Due To**

Specimens that have been decalcified (all methods) Specimens that have not been formalin-fixed, paraffin-embedded

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**Specimen Minimum Volume**

See Specimen Required

**Specimen Stability Information**

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

## Clinical & Interpretive

### Clinical Information

Targeted cancer therapies are defined as antibody or small molecule drugs that block the growth and spread of cancer by interfering with specific cell molecules involved in tumor growth and progression. Multiple targeted therapies have been approved by the FDA for treatment of specific cancers. Molecular genetic profiling is often needed to identify targets amenable to targeted therapies and to minimize treatment costs and therapy-associated risks.

Next generation sequencing has recently emerged as an accurate, cost-effective method to identify mutations across numerous genes known to be associated with response or resistance to specific targeted therapies. The results of this test can be useful for assessing prognosis and guiding treatment of individuals with solid tumors. These data can also be used to help determine clinical trial eligibility for patients with mutations in genes not amenable to current FDA-approved targeted therapies.

The epidermal growth factor receptor (EGFR) gene product is activated by the binding of specific ligands (epiregulin and amphiregulin), resulting in activation of the RAS/MAPK pathway. Activation of this pathway induces a signaling cascade ultimately regulating a number of cellular processes including cell proliferation. Dysregulation of the RAS/MAPK pathway is a key factor in tumor progression. Targeted therapies directed to EGFR, which inhibit activation of the RAS/MAPK pathway, have demonstrated some success (increased progression-free and overall survival) in patients with colorectal cancer.

Assessment for *BRAF* mutations has clinical utility in that it is a predictor of response to antimutant BRAF therapy. BRAF is a member of the mitogen-activated protein/extracellular signal-regulated (MAP/ERK) kinase pathway, which plays a role in cell proliferation and differentiation. Dysregulation of this pathway is a key factor in tumor progression. Targeted therapies directed to components of this pathway have demonstrated some success with increases both in progression-free and overall survival in patients with certain tumors. Effectiveness of these therapies, however, depends in part on the mutation status of the pathway components.

See [Targeted Gene Regions Interrogated by RAS/RAF Gene Panel](#) in Special Instructions for details regarding the targeted gene regions identified by this test.

### Reference Values

An interpretive report will be provided.

**Interpretation**

An interpretive report will be provided.

**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 (wild-type) result does not rule out the presence of a mutation that may be present but below the limits of detection of this assay (approximately 5%-10%).

This test does not detect large single or multiexon deletions or duplications or genomic copy number variants.

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 findings, tumor sampling, and other laboratory data. If results obtained do not match other clinical or laboratory findings, please contact the laboratory for updated interpretation. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

**Clinical Reference**

1. Targeted Cancer Therapies. National Cancer Institute Fact Sheet. Updated 12/05/2012. Accessed December 2013, Available at [www.cancer.gov/cancertopics/factsheet/Therapy/targeted](http://www.cancer.gov/cancertopics/factsheet/Therapy/targeted)
2. Vogelstein B, Papadopoulos N, Velculescu VE, et al: Cancer genome landscapes. *Science* 2013;339:1546-1558
3. Beadling C, Neff TL, Heinrich MC, et al: Combining highly multiplexed PCR with semiconductor-based sequencing for rapid cancer genotyping. *J Mol Diagn* 2013;15:171-176
4. Anderson S, Bloom KJ, Vallera DU, et al: Multisite analytic performance studies of a real-time polymerase chain reaction assay for the detection of BRAF V600E mutations in formalin-fixed paraffin-embedded tissue specimens of malignant melanoma. *Arch Pathol Lab Med* 2012 Feb;136:1-7
5. Di Nicolantonio F, Martini M, Molinari F, et al: Wild-type BRAF is required for response to Panitumumab or Cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008;26:5705-5712
6. Flaherty KT, Puzanov I, Kim KB, et al: Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 2010;363(9):809-819

7. Ladanyi M, Pao W: Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. *Mod Pathol* 2008;21 Suppl 2:S16-S22

8. Lievre A, Bachet JB, Le Corre D, et al: KRAS mutation status is predictive of response to Cetuximab therapy in colorectal cancer. *Cancer Res* 2006;66(8):3992-3995

## Performance

### Method Description

Next-generation sequencing is performed to test for the presence of a mutation in targeted regions of the following 4 genes: *BRAF*, *HRAS*, *NRAS*, *KRAS*. (Unpublished Mayo method)

See [Targeted Gene Regions Interrogated by RAS/RAF Gene Panel](#) in Special Instructions for details regarding the targeted gene regions identified by this test.

### PDF Report

No

### Specimen Retention Time

Unused portions of blocks will be returned. Unused slides are stored indefinitely.

### Performing Laboratory Location

Rochester

## Fees & Codes

### 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

RAS/RAF Targeted Gene Panel by Next Generation Sequencing, Tumor

81210-*BRAF* (v-raf murine sarcoma viral oncogene homolog B1) (eg, colon cancer), gene analysis, V600E variant

81275-*KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene) (eg, carcinoma) gene analysis, variants in codons 12 and 13

81403-*HRAS* (v-Ha-ras Harvey rat sarcoma viral oncogene homolog) (eg, Costello syndrome), exon 2 sequence

81311-*NRAS* (neuroblastoma RAS viral [v-ras] oncogene homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)

Slide Review

88381-Microdissection, manual

## LOINC® Information

Test ID	Test Order Name	Order LOINC Value
RASFP	RAS/RAF Panel, Tumor	In Process

Result ID	Reporting Name	LOINC®
36725	Result Summary	50397-9
36726	Result	82939-0
36727	Interpretation	69047-9
36728	Additional Information	48767-8
36729	Specimen	31208-2
36730	Source	33725-3
36731	Tissue ID	80398-1
36732	Released By	18771-6