MAYO CLINIC LABORATORIES

# Test Definition: TPNUQ

Thiopurine Methyltransferase (TPMT) and Nudix Hydrolase (NUDT15) Genotyping, Varies

## Overview

### Useful For

Predicting potential for toxicity to thiopurine drugs (6-mercaptopurine, 6-thioguanine, and azathioprine)

### Testing Algorithm

For information see:

-Ulcerative Colitis and Crohn Disease Therapeutic Drug Monitoring Algorithm

-TPMT Testing in the Treatment of Inflammatory Bowel Disease Algorithm

### Special Instructions

- Informed Consent for Genetic Testing
- Pharmacogenomic Association Tables
- Multiple Genotype Test List
- Informed Consent for Genetic Testing (Spanish)
- Ulcerative Colitis and Crohn Disease Therapeutic Drug Monitoring Algorithm
- TPMT Testing in the Treatment of Inflammatory Bowel Disease Algorithm

### Highlights

This test includes genotyping of *TPMT* and *NUDT15*, both of which affect metabolism of thiopurine drugs.

### Method Name

Real-Time Polymerase Chain Reaction (PCR) With Allelic Discrimination Analysis

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Ordering Guidance

For thiopurine methyltransferase (TPMT) enzyme activity testing, order TPMT3 / Thiopurine Methyltransferase Activity Profile, Erythrocytes; however, this test should also be ordered because TPMT enzyme activity testing cannot detect variants in *NUDT15*, which also impact thiopurine metabolism.

### Specimen Required

**Submit only 1 of the following specimens:**

**Patient Preparation:** A previous hematopoietic stem cell transplant from an allogenic donor will interfere with testing.

Call 800-533-1710 for instructions for testing patients who have received a hematopoietic stem cell transplant.
**Specimen Type**: Whole blood
**Container/Tube**: Lavender top (EDTA)
**Specimen Volume**: 3 mL
**Collection Instructions**:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**.
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information
**Specimen Stability Information**: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
**Additional Information**:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA is met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

**Specimen Type:** Saliva
**Patient Preparation:** Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.
**Supplies:**
DNA Saliva Kit High Yield (T1007)
Saliva Swab Collection Kit (T786)
**Container/Tube:**
**Preferred**: High-yield DNA saliva kit
**Acceptable**: Saliva swab
**Specimen Volume**: 1 Tube if using T1007 or 2 swabs if using T786
**Collection Instructions:** Collect and send specimen per kit instructions.
**Specimen Stability Information:** Ambient (preferred) 30 days/Refrigerated 30 days
**Additional Information:** Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

**Specimen Type**: Extracted DNA
**Container/Tube**:
**Preferred**: Screw Cap Micro Tube, 2 mL with skirted conical base
**Acceptable**: Matrix tube, 1 mL
**Collection Instructions**:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
**Specimen Stability Information**: Frozen (preferred) 1 year/Ambient/Refrigerated
**Additional Information**: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from

a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

### Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-Neurology Specialty Testing Client Test Request (T732)
-Gastroenterology and Hepatology Test Request (T728)
-Therapeutics Test Request (T831)

### 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 | Varies | | |

## Clinical & Interpretive

### Clinical Information

The thiopurine drugs are purine antimetabolites that are useful in the treatment of acute lymphoblastic leukemia, autoimmune disorders (eg, Crohn disease, rheumatoid arthritis), and organ transplant recipients. The thiopurine drugs, 6-mercaptopurine, 6-thioguanine, and azathioprine are prodrugs that require intracellular activation to 6-thioguanine nucleotides (6-TGN). This activation is catalyzed by multiple enzymes. The cytotoxic effects of thiopurine drugs are achieved mainly through incorporation of 6-TGN into DNA and RNA. The pathway that leads to synthesis of active cytotoxic 6-TGN is in competition with inactivation pathways catalyzed by thiopurine methyltransferase (TPMT). Evaluation of this pathway is important because the level of 6-TGN measured in red blood cells have been correlated with both thiopurine therapeutic efficacy and toxicity such as myelosuppression.

TPMT activity is inherited as a monogenic codominant trait, and variable TPMT activity is associated with *TPMT* genetic variants. The distribution of TPMT activity in red blood cells is trimodal in the population of people with European ancestry, with approximately 0.3% having deficient (undetectable) TPMT activity, 11% low (intermediate) activity, and 89% normal TPMT activity. The allele for normal TPMT activity (wild type) has been designated *TPMT*1*. Four *TPMT*

alleles, comprised of a combination of 3 different single-nucleotide variants, account for the majority of inactivating alleles in some ancestral populations, including Europeans: *TPMT*2*, *TPMT*3A*, and *TPMT*3C*. Less frequently occurring *TPMT* alleles including *TPMT*4*, *TPMT*5*, *TPMT*8*, and *TPMT*12* also have been implicated as deficiency alleles. If no *TPMT* variant alleles are detected by this assay, the most likely genotype is that of *TPMT*1/*1*, although the presence of other rarer alleles cannot be excluded.

Nudix hydrolase (NUDT15) is thought to dephosphorylate the active metabolites of thiopurines, TGTP, and TdGTP, which prevents their incorporation into DNA and decreases their cytotoxic effects. Genetic variants in *NUDT15* that decrease this activity are strongly associated with thiopurine-related myelosuppression. NUDT15 deficiency is most common among East Asian (22.6%), South Asian (13.6%), and Native American populations (12.5%-21.2%). Studies in other populations are ongoing. This test evaluates variants associated with *NUDT15*2, NUDT15*3, NUDT15*4,* and *NUDT15 *5*. If no *NUDT15* variant alleles are detected by this assay, the most likely genotype is that of *NUDT15*1/*1*, although the presence of other rarer alleles cannot be excluded. Individuals with variants in both *TPMT* and *NUDT15* have been identified and were significantly more sensitive to mercaptopurine than individuals heterozygous for a variant in only one gene. Integration of both *TPMT* and *NUDT15* testing may allow for more accurate prediction of thiopurine-related toxicity risk to guide dosing, particularly among patients from diverse populations.

Table 1. TPMT Enzyme Activity of Individual Star Alleles

| ***TPMT* allele** | **cDNA nucleotide change (NM_000367.4)** | **Amino acid change** | **Effect on enzyme metabolism** |
|---|---|---|---|
| **1* | None (wild type) | None (wild type) | Normal function |
| **2* | c.238G>C | p.Ala80Pro (p.A80P) | No activity |
| **3A* | c.460G>A and c.719A>G | p.Ala154Thr (p.A154T) and p.Tyr240Cys (p.Y240C) | No activity |
| **3B* | c.460G>A | p.Ala154Thr (p.A154T) | No activity |
| **3C* | c.719A>G | p.Tyr240Cys (p.Y240C) | No activity |
| **4* | c.626-1G>A | Not applicable, splice site | No activity |
| **5* | c.146T>C | p.Leu49Ser (p.L49S) | No activity |
| **8* | c.644G>A | p.Arg215His (p.R215H) | Reduced activity |
| **12* | c.374C>T | p.Ser125Leu (p.S125L) | Reduced activity |

Table 2. NUDT15 Enzyme Activity of Individual Star Alleles

| ***NUDT15* allele** | **cDNA nucleotide change (NM_018283.3)** | **Amino acid change** | **Effect on enzyme metabolism** |
|---|---|---|---|
| **1* | None (wild type) | None (wild type) | Normal activity |
| **2* or **3* | c.415C>T | p.Arg139Cys (p.R139C) | No activity |
| **4* | c.416G>A | p.Arg139His (p.R139H) | No activity |
| **5* | c.52G>A | p.Val18Ile (p.V18I) | No activity |

The US Food and Drug Administration, the Clinical Pharmacogenetics Implementation Consortium, and some professional societies recommend consideration of *TPMT* and *NUDT15* genotype testing or TPMT enzyme activity testing along with *NUDT15* genotype testing prior to the initiation of therapy with thiopurine drugs. There is substantial

evidence linking *TPMT* and *NUDT15* genotypes to phenotypic variability. Dose adjustments based upon *TPMT* and *NUDT15* genotypes have reduced thiopurine-induced adverse effects without compromising desired antitumor and immunosuppressive therapeutic effects in several clinical settings.

Genotyping is not impacted by other medications known to inhibit TPMT activity. Complementary clinical testing is available to measure TPMT enzymatic activity in erythrocytes (TPMT3 / Thiopurine Methyltransferase Activity Profile, Erythrocytes) if the clinician wants to check for lower TPMT enzyme activity, regardless of cause. Testing for TPMT enzyme activity is not impacted by variants in *NUDT15*.

### Reference Values

An interpretive report will be provided.

### Interpretation

The *TPMT* genotype, with associated star alleles, is assigned using standard allelic nomenclature as published by the TPMT Nomenclature Committee.(1) *NUDT15* genotype and associated star alleles are as described by Moriyama et al.(2) and catalogued in the Pharmacogene Variation Consortium (www.pharmvar.org).

For additional information regarding pharmacogenomic genes and their associated drugs, see the Pharmacogenomics Associations Tables. This resource also includes information regarding enzyme inhibitors and inducers, as well as potential alternate drug choices.

### Cautions

Rare variants may be present that could lead to false-negative or false-positive results. If no *TPMT* variant alleles are detected by this assay, the most likely genotype is that of *TPMT*1/*1* although the presence of other rarer alleles cannot be excluded. In addition, if no *NUDT15* variant alleles are detected by this assay, the most likely genotype is that of *NUDT15*1/*1*, although the presence of other rarer alleles cannot be excluded.

If genotype results obtained do not match the clinical findings, additional testing should be considered for thiopurine methyltransferase enzyme activity (TPMT3 / Thiopurine Methyltransferase Activity Profile, Erythrocytes). A corresponding activity assay for NUDT15 is not currently available.

Specimens may contain donor DNA if obtained from patients who received non-leukoreduced blood transfusions or allogeneic hematopoietic stem cell transplantation. Results from specimens obtained under these circumstances may not accurately reflect the recipient's genotype. For individuals who have received blood transfusions, the genotype usually reverts to that of the recipient within 6 weeks. For individuals who have received allogeneic hematopoietic stem cell transplantation, a pretransplant DNA specimen is recommended for testing.

The results do not rule out the possibility that a patient harbors another variant in *TPMT*, *NUDT15*, or another gene that can impact drug response or side effects. These genotyping procedures will not distinguish between *TPMT *1/*3A* from the rare *TPMT *3B/*3C* genotype, which has an estimated frequency of 1:120,890. This rare genotype is associated with low enzyme activity. Enzyme activity evaluation is necessary to definitively identify this rare genotype (TPMT3 / Thiopurine Methyltransferase Activity Profile, Erythrocytes).

This test will not detect all *TPMT* or *NUDT15* genetic variants. A negative result does not rule out the possibility of toxicity if thiopurines are used, since multiple factors (eg, other genetic factors, drug-drug interactions) are known to

play a role. Co-prescription of allopurinol might inhibit TPMT activity. Other drugs that have been shown to inhibit TPMT activity include naproxen, ibuprofen, ketoprofen, furosemide, sulfasalazine, mesalamine, olsalazine, mefenamic acid, thiazide diuretics, and benzoic acid inhibitors.

### Clinical Reference

1. TPMT nomenclature committee (TPMT Alleles): Table of TPMT Alleles. Linkoping University; Updated November 2022. Accessed March 19, 2025. Available at https://liu.se/en/research/tpmt-nomenclature-committee
2. Moriyama T, Nishii R, Perez-Andreu V, et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet. 2016;48(4):367-373. doi:10.1038/ng.3508
3. Appell ML, Berg J, Duley J, et al. Nomenclature for alleles of the thiopurine methyltransferase gene. Pharmacogenet Genomics. 2013;23(4):242-248. doi:10.1097/FPC.0b013e32835f1cc0
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6. Weinshilboum R. Thiopurine pharmacogenetics: clinical and molecular studies of thiopurine methyltransferase. Drug Metab Dispos. 2001;29(4 Pt 2):601-605
7. Zaza G, Cheok M, Krynetskaia N, et al. Thiopurine pathway. Pharmacogenet Genomics. 2010;20(9):573-574. doi:10.1097/FPC.0b013e328334338f
8. Sterner RM, Hall PL, Matern D, Black JL, Moyer AM. Genotype and Phenotype Correlation of the TPMT*8 Allele in Thiopurine Metabolism. J Mol Diagn. 2024;26(11):988-994. doi:10.1016/j.jmoldx.2024.07.005
9. Pratt VM, Cavallari LH, Fulmer ML, et al. TPMT and NUDT15 Genotyping Recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase. J Mol Diagn. 2022;24(10):1051-1063. doi:10.1016/j.jmoldx.2022.06.007

## Performance

### Method Description

Genomic DNA is extracted from whole blood or saliva. Genotyping for the *TPMT* and *NUDT15* alleles is performed using a polymerase chain reaction (PCR)-based 5'-nuclease assay. Fluorescently labeled detection probes anneal to the target DNA. PCR is used to amplify the segment of DNA that contains the polymorphism. If the detection probe is an exact match to the target DNA, the 5'-nuclease polymerase degrades the probe, the reporter dye is released from the effects of the quencher dye, and a fluorescent signal is detected. Genotypes are assigned based on the allele-specific fluorescent signals that are detected.(Unpublished Mayo method)

### PDF Report

No

### Day(s) Performed

Monday through Friday

### Report Available
2 to 4 days

### Specimen Retention Time
Whole blood/Saliva: 30 days (if available); 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
0034U

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| TPNUQ | TPMT and NUDT15 Genotype, V | 93193-1 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 610159 | TPMT Genotype | 41048-0 |
| 610160 | TPMT Phenotype | 79713-4 |
| 610161 | NUDT15 Genotype | 93194-9 |
| 610162 | NUDT15 Phenotype | 93195-6 |
| 610163 | Interpretation | 69047-9 |
| 610164 | Additional Information | 48767-8 |
| 610165 | Method | 85069-3 |
| 610166 | Disclaimer | 62364-5 |
| 610167 | Reviewed by | 18771-6 |