MAYO CLINIC LABORATORIES

# Test Definition: WBSEQ

Beta Globin Gene Sequencing, Varies

## Overview

### Useful For

Diagnosis of beta thalassemia intermedia or major

Identification of a specific beta thalassemia sequence variant (ie, unusually severe beta thalassemia trait)

Evaluation of an abnormal hemoglobin electrophoresis identifying a rare beta-globin variant

Evaluation of chronic hemolytic anemia of unknown etiology

Evaluation of hereditary erythrocytosis with left-shifted p50 oxygen dissociation results

Preconception screening when there is a concern for a beta-hemoglobin disorder based on family history

### Genetics Test Information

Beta-globin gene (*HBB*) sequencing can be used to identify hemoglobin variants and the most common beta thalassemia sequence variants, including beta plus and beta zero thalassemias. It also identifies hyper-unstable hemoglobin variants and dominant beta thalassemia sequence variants, as well as other hemoglobin variants that cannot be identified by protein methods. Some hemoglobin disorders will not be detected by beta-globin gene sequencing, such as large deletional alterations and crossover events. As such, the results of this test should always be interpreted within the context of the protein studies and red blood cell indices.

### Testing Algorithm

A hemoglobin electrophoresis evaluation (HBEL1 / Hemoglobin Electrophoresis Evaluation, Blood) is always indicated prior to beta-globin gene sequencing because these conditions can be complex and protein data allows accurate and rapid classification of the patient phenotype.

### Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)

### Highlights

This is a second-tier evaluation of beta thalassemia minor, intermedia, and major, as well as beta-globin variant identification.

### Method Name

Polymerase Chain Reaction (PCR)/Sanger Sequencing

### NY State Available

Yes

## Specimen

### Specimen Type
Varies

### Ordering Guidance
For first-tier testing for beta thalassemia, order THEV1 / Thalassemia and Hemoglobinopathy Evaluation, Blood and Serum.

For first-tier testing for beta-globin variant detection, order HBEL1 / Hemoglobin Electrophoresis Evaluation, Blood.

### Necessary Information
**1. Patient's age is required.**
2. Include recent transfusion information.

### Specimen Required
**Specimen Type:** Whole blood
**Container/Tube:**
**Preferred:** Lavender top (EDTA)
**Acceptable:** Yellow top (ACD), green top (sodium heparin)
**Specimen Volume:** 4 mL
**Collection Instructions:**
1. Invert several times to mix whole blood.
2. Send whole blood specimen in the original tube. **Do not aliquot.**
**Specimen Stability Information:** Refrigerate 30 days(preferred)/Ambient 14 days

**Specimen Type:** Extracted DNA from whole blood
**Container/Tube:** 1.5- to 2-mL tube
**Specimen Volume:** Entire specimen
**Collection Instructions:**
1. DNA must be extracted from whole blood within 7 days of collection.
2. Label specimen as extracted DNA and source of specimen.
3. Provide volume and concentration of the DNA.
**Specimen Stability Information:** Frozen (preferred) 1 year/Refrigerate/Ambient
**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). We 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.

### 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. Metabolic Hematology Patient Information (T810)
3. If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen.

### Specimen Minimum Volume

Whole blood: 1 mL; Extracted DNA: 50 mcL at 50 ng/mcL concentration

### Reject Due To

| | |
|---|---|
| Moderately to severely clotted | Reject |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Varies | Varies | | |

## Clinical & Interpretive

### Clinical Information

Beta-globin gene sequencing is useful in the evaluation of beta-globin chain variants and beta thalassemia. It detects almost all beta-globin variants and the most common beta thalassemia sequence variants, although prevalence is ethnicity dependent. Because these conditions are often complex, this test should always be interpreted in the context of protein studies, such as hemoglobin electrophoresis and red blood cell indices.

The majority of beta-globin chain variants are clinically and hematologically benign; however, some have important clinical consequences, such as erythrocytosis, cyanosis/hypoxia, chronic hemolysis, or unexplained microcytosis. Most of the common clinically significant hemoglobin (Hb) variants (ie, HbS, HbC, HbE, and others) are easily distinguished by hemoglobin electrophoresis and do not require molecular analysis. In addition, they are frequently found in complex hemoglobin disorders due to multiple genetic variants, and accurate classification requires sequencing data within the context of protein data. In some instances, beta-globin sequencing is necessary to identify or confirm the identity of rare variants, especially those associated with erythrocytosis and chronic hemolytic anemia. Rare hyper-unstable variants (also termed dominant beta thalassemia mutations) result in hemolytic anemia and do not create protein stable enough to be detectable by protein methods, including stability studies. They are not always associated with elevated HbA2 or microcytosis and, therefore, can be electrophoretically silent. These require a high degree of clinical suspicion as all electrophoretic testing as well as stability studies cannot exclude this condition.

Beta thalassemia is an autosomal recessive condition characterized by decreased or absent synthesis of beta-globin chains due to sequence variants in the beta-globin gene (*HBB)*. No abnormal protein is present and diagnosis by electrophoresis relies on hemoglobin fraction percentage alterations (ie, HbA2 or HbF elevations).

Beta thalassemia can be split into 3 broad classes (categorized by clinical features):
1. Beta thalassemia trait (also called beta thalassemia minor and beta thalassemia carrier) (B[A]B[+] or B[A]B[0])
2. Beta thalassemia intermedia (B[+]B[+] or B[+]B[0])

3. Beta thalassemia major (B[+]B[0] or B[0]B[0])

Beta thalassemia trait is typically a harmless condition with varying degrees of microcytosis and hypochromia and sometimes mild anemia. Transfusions are not required. Beta thalassemia intermedia is a clinical distinction and is characterized by a more severe degree of anemia than beta thalassemia trait with few or intermittent transfusions required. Later in life, these individuals are at risk for iron overload even in the absence of chronic transfusion due to increased intestinal absorption of iron. Beta thalassemia major typically comes to medical attention early in life due to severe anemia, hepatosplenomegaly, and failure to thrive. Skeletal changes are also common due to expansion of the bone marrow. Without appropriate treatment these patients have a shortened lifespan.

The majority of beta thalassemia variations (>90%) are point alterations, small deletions, or insertions, which are detected by beta-globin gene sequencing. The remaining beta thalassemia sequence variants are either due to large genomic deletions of *HBB* or, very rarely, trans-acting beta thalassemia variations located outside of the beta-globin gene cluster. Some rare beta-chain variants can be clinically or electrophoretically indistinguishable from beta thalassemia and cannot be confirmed without molecular analysis.

## Reference Values

An interpretive report will be provided.

## Interpretation

The alteration will be provided with the classification, if known. Further interpretation requires correlation with protein studies and red blood cell indices.

## Cautions

This assay will not detect large deletions or duplications within the beta globin gene. In addition, hybrid beta globin variants (ie, hemoglobin Lepore) will not be detected by this method. This method cannot distinguish between homozygous and compound heterozygous variants associated with large deletions. This method cannot distinguish between double substitution on single chromosome and a compound heterozygous state. Beta-globin sequencing alone is not able to distinguish between alterations that are found in the same copy of the *HBB* gene (ie, variants that are "linked" or "in cis") and alterations found on different *HBB* gene copies (ie, are "in trans"). This limitation of sequencing may complicate diagnosis and has implications for inheritance and proper genetic counseling. To resolve these cases, molecular results must be correlated with electrophoretic and protein data, other laboratory findings, clinical findings, and family studies. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Rare genetic alterations exist that could lead to false-negative or false-positive results. If results obtained do not match the clinical findings, additional testing should be considered.

## Clinical Reference

1. Hoyer JD, Hoffman DR. The thalassemia and hemoglobinopathy syndromes. In: McClatchey KD, ed. Clinical Laboratory Medicine. 2nd ed. Lippincott Williams and Wilkins; 2002:866-895
2. Thein SL. The molecular basis of beta-thalassemia. Cold Spring Harb Perspect Med. 2013;3(5):a011700
3. Hoyer JD, Kroft, SH. Color Atlas of Hemoglobin Disorders: A Compendium Based on Proficiency Testing. CAP; 2003
4. Merchant S, Oliveira JL, Hoyer JD, Viswanatha DS. Molecular diagnosis in hematopathology. In: Hsi E, Volume ed. Goldblum J, ed. Hematopathology: A Volume in Foundations in Diagnostic Pathology Series. 2nd ed. Churchill Livingstone; 2012
5. Rangan A, Hein MS, Jenkinson WG, et al. Improved characterization of complex beta-globin gene cluster structural

variants using long-read sequencing. J Mol Diagn. 2021;23(12):1732-1740. doi:10.1016/j.jmoldx.2021.08.013
6. Oliveira JL, Thompson CH, Saravanaperumal SA, et al. epsilon-gamma-thalassemia, a new hemoglobinopathy category. Clin Chem. 2023;69(7):711-717. doi:10.1093/clinchem/hvad038

## Performance

### Method Description
Genomic DNA is extracted from whole blood. The *HBB* gene is amplified by polymerase chain reaction (PCR). The PCR product is then purified and sequenced in both directions using fluorescent dye-terminator chemistry. Sequencing products are separated on an automated sequencer and trace files analyzed for variations in all exons, introns with the exception of IVS-II-82 through IVS-II-650, the 5'UTR, the 3'UTR, and the promoter region. Results are correlated with routine studies to identify unusual beta globin variants.(Reddy PL, Bowie LJ. Sequence-based diagnosis of hemoglobinopathies in the clinical laboratory. Clin Lab Med. 1997;17[1]:85-96; Traeger-Synodinos J, Harteveld CL. Advances in technologies for screening and diagnosis of hemoglobinopathies. Biomarkers Med. 2014;8[1]:115-127)

### PDF Report
No

### Day(s) Performed
Monday through Friday

### Report Available
10 days

### Specimen Retention Time
Whole blood: 2 weeks; 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

81364-HBB (hemoglobin, beta) full sequence

## LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| WBSEQ | Beta Globin Gene Sequencing, B | 79401-6 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 62128 | Beta Globin Gene Sequencing, B | 82939-0 |
| 43922 | Interpretation | 69047-9 |