

# Thalassemia StripAssays®

The easy way to optimize thalassemia screening using established innovations in diagnostics

## Thalassemia Assays Key to efficient screening

Thalassemias are a major public health problem, particularly in Mediterranean countries, the Middle East, Asia, India and parts of Africa. For the large majority of affected individuals there is only supportive management but no ultimate cure. Health authorities therefore focus on prevention programs based on heterozygous carrier screening and prenatal diagnosis. Since only a few  $\alpha$ - and  $\beta$ -globin alleles are prevalent in each at-risk population, large-scale screening programs are feasible, but require simple and automated test procedures.

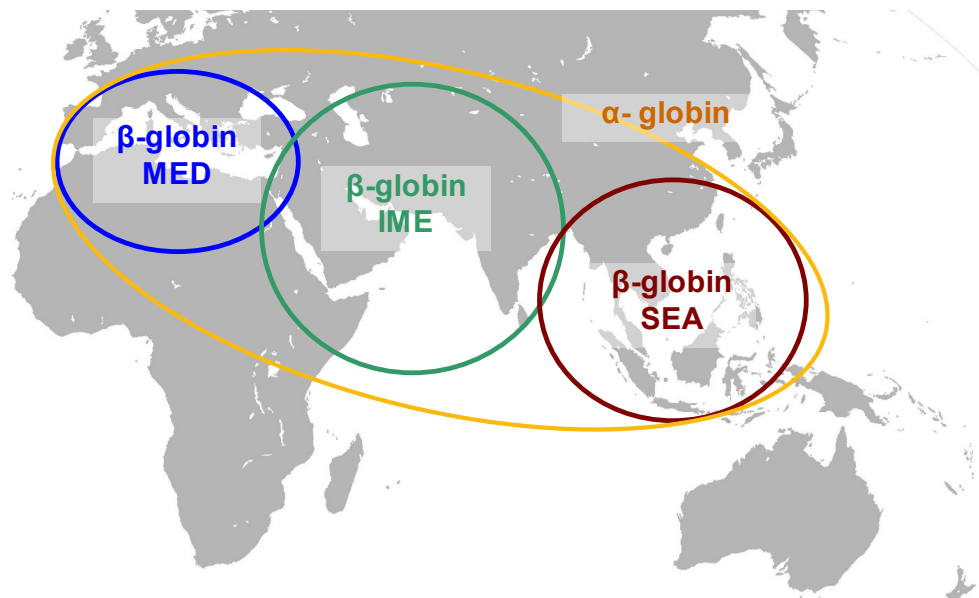
In healthy adults 97-98% of total hemoglobin (Hb) is HbA, consisting of two  $\alpha$ -globin and two  $\beta$ -globin polypeptides ( $\alpha_2\beta_2$ ). Abnormalities in the structure and synthesis of both globin chains lead to an imbalance causing the two main types of thalassemia:  $\alpha$ -thalassemia and  $\beta$ -thalassemia.

The loss of one of the two  $\alpha$ -globin alleles ( $-\alpha$ ) on chromosome 16 causes  $\alpha^+$ -thalassemia, whereas  $\alpha^0$ -thalassemia is due to inactivation of both  $\alpha$ -globin alleles ( $--$ ).

Two groups of  $\beta$ -globin mutations are distinguished, depending on whether they lead to a reduction ( $\beta^+$ ) or an absence ( $\beta^0$ ) of  $\beta$ -globin synthesis.

In many regions,  $\alpha$ - and  $\beta$ -thalassemia coexist with a variety of different structural Hb variants. These complex interactions give rise to an extremely wide spectrum of clinical phenotypes. Furthermore, an inherited increase of  $\gamma$ -globin expression in patients can partially compensate for the lack of normal  $\beta$ -globin chain synthesis in  $\beta$ -thalassemia as well as sickle cell disease and thereby can ameliorate the clinical phenotype of both disorders.

ViennaLab offers the globally applicable  $\alpha$ -globin StripAssay®, three tailored  $\beta$ -globin StripAssays® for the Mediterranean area (MED), India & Middle East (IME) and Southeast Asia (SEA), as well as the  $\beta$ -Thal Modifier StripAssay®



# $\beta$ -Globin

Numerous defects in the  $\beta$ -globin gene have been identified, many of which cause structural abnormalities, such as HbS (sickle cell hemoglobin), HbC, HbE, or lead to impaired  $\beta$ -globin synthesis, known as  $\beta$ -thalassemia. The clinical manifestations of the disease show a tremendous diversity, which correlates with the underlying genetic defects.  $\beta$ -Thalassemia major, the most severe and transfusion-dependent state is characterized by the complete absence of HbA and results from the inheritance of two  $\beta^0$  alleles. Individuals who have inherited a single  $\beta$ -thalassemia allele, whether  $\beta^0$  or  $\beta^+$ , have  $\beta$ -thalassemia minor. They are often clinically asymptomatic but may have mild anemia with characteristic hypochromic microcytic red blood cells and elevated levels of HbA<sub>2</sub> ( $\alpha_2\delta_2$ ). The diverse phenotypes ranging between  $\beta$ -thalassemia major and  $\beta$ -thalassemia minor constitute the clinical syndrome of  $\beta$ -thalassemia intermedia, which in most cases results from the inheritance of either  $\beta^+/\beta^+$  or  $\beta^+/\beta^0$  alleles.

Position	Sequence alteration	$\beta$ -thal type	MED 4-130	IME 4-140	SEA 4-150
- 101	C>T	+	x		
- 87	C>G	+	x		
- 31	A>G	+			x
- 30	T>A	+	x		
- 29	A>G	+			x
- 28	A>G	+			x
cap+1	A>C	+		x	x
initiation cd	ATG>AGG	0			x
cd 5	-CT	0	x	x	
cd 6	G>A (HbC)	-	x		
cd 6	A>T (HbS)	-	x	x	
cd 6	-A	0	x		
cd 8	-AA	0	x	x	
cd 8/9	+G	0	x	x	x
cd 15	TGG>TGA	0	x		
cd 15	TGG>TAG	0		x	x
cd 16	-C	0		x	
cd 17	A>T	0			x
cd 19	A>G (Hb Malay)	+			x
cd 22	7bp deletion	0		x	
cd 26 HbE	G>A	-			x
cd 27	G>T (Hb Knossos)	+	x		
cd 27/28	+C	0			x
cd 30	G>C	0		x	
IVS 1.1	G>A	0	x	x	
IVS 1.1	G>T	0		x	x
IVS 1.5	G>C	+	x	x	x
IVS 1.6	T>C	+	x	x	
IVS 1.110	G>A	+	x	x	
IVS 1.116	T>G	0	x		
IVS 1.130	G>C	0	x		
IVS 1-25	25bp deletion	0		x	
cd 36/37	-T	0		x	
cd 39	C>T	0	x	x	
cd 41/42	-TTCT	0		x	x
cd 43	G>T	0			x
cd 44	-C	0	x	x	
cd 71/72	+A	0			x
cd 89/90	-GT	0			x
cd 90	G>T	0			x
cd 95	+A	0			x
IVS 2.1	G>A	0	x	x	
IVS 2.654	C>T	+			x
IVS 2.745	C>G	+	x	x	
IVS 2.848	C>A	+	x		
cd 121	G>T	0			x
619bp del	exon 3 deletion	0		x	

## $\beta$ -Globin StripAssays®

ViennaLab offers reliable and convenient reverse-hybridization assays tailored to population-specific mutations in different regions.

$\beta$ -Globin StripAssay® MED

4-130

22 mutations covering >90% of  $\beta$ -globin defects found in Mediterranean countries

$\beta$ -Globin StripAssay® IME

4-140

22 mutations covering >90% of  $\beta$ -globin defects found in the Middle East and India

$\beta$ -Globin StripAssay® SEA

4-150

22 mutations covering >90% of  $\beta$ -globin defects found in Southeast Asia

# α-Globin

Reduced or absent α-globin synthesis, mainly caused by deletions of one or both α-globin genes (α<sub>1</sub>, α<sub>2</sub>) and less frequently by point mutations, leads to α-thalassemia. The severity of the phenotype depends on the number of affected α-globin genes and the resulting imbalance between α- and β-globin chains. Four intact α-globin genes (αα/αα) are present in the diploid genome of a healthy human. Individuals, who inherit only two or three functional genes, have mild anemia and microcytosis. HbH disease, affecting only subjects with a single active α-globin gene, presents with moderate to severe hemolytic anemia. The most severe manifestation, the loss of all four α-globin genes (---), cause homozygous α<sup>0</sup>-thalassemia (Hb Bart's hydrops fetalis syndrome), which is generally associated with death *in utero*.

## α-Globin StripAssay®

ViennaLab offers a reliable and convenient reverse-hybridisation assay, which is capable of analyzing simultaneously common α-globin single and double gene deletions, small deletions, point mutations and gene triplication by a single approach.

α-Globin StripAssay®

4-160

21 mutations covering >90% of α-globin defects found in Mediterranean, Middle Eastern and Southeast Asian countries

The Assay

Mutations covered by the ViennaLab  
α-Globin StripAssay®  
using two separate teststrips (A/B) per sample

Position	Sequence alteration	Test strip
-α <sup>3,7</sup>	single gene deletion	A
-α <sup>4,2</sup>	single gene deletion	A
-(α) <sup>20,5</sup>	double gene deletion	A
--MED	double gene deletion	A
--SEA	double gene deletion	A
--THAI	double gene deletion	A
--FIL	double gene deletion	A
α1 cd 14	G>A	A
α1 cd 59	G>A (Hb Adana)	A
ααα <sup>emb-3,7</sup>	gene triplication	B
α2 initiation cd	ATG>ACG	B
α2 cd 19	-G	B
α2 IVS1	5bp deletion	B
α2 cd 59	G>A	B
α2 cd 125	T>C (Hb Quong Sze)	B
α2 cd 142	T>C (Hb Constant Spring)	B
α2 cd 142	T>A (Hb Icaria)	B
α2 cd 142	A>T (Hb Pakse)	B
α2 cd 142	A>C (Hb Koya Dora)	B
α2 polyA-1	AATAAA>AATAAG (Saudi type)	B
α2 polyA-2	AATAAA>AATGAA (Turkish type)	B

# β-Thal Modifier

Despite identical genotypes, the clinical manifestation of β-thalassemia and sickle cell disease can be highly variable between individual patients. The clinical phenotype ranges from a severe transfusion-dependent condition to a relatively mild clinical course. Apart from silent or mild β-thalassemia mutations and co-inheritance of α-thalassemia, genetic determinants leading to continuous production of γ-globin chains in adult life play an important role in modifying disease severity. Hemoglobin F (HbF: α<sub>2</sub>γ<sub>2</sub>) is the principal factor ameliorating the severity of both disorders by reducing the α-/β-globin chain imbalance. The level of HbF expression in adults, inherited as a quantitative trait, has emerged as an important prognostic factor in sickle-cell disease and β-thalassemia major. The three major quantitative trait loci in the *HBG2*, *BCL11A* and *HBS1L-MYB* genes account for 20% to 50% of the common variation in HbF levels and are considered to be the best predictors of disease severity in β-hemoglobinopathies.

## β-Thal Modifier StripAssay®

- assists in anticipating the type of β-thalassemia - major or intermedia - in an early phase
- helps in predicting the clinical course of β-thalassemia and sickle cell disease in newborns
- supports therapeutic decision making regarding, for example, hematopoietic stem cell transplantation
- rises accuracy of genetic counseling

Polymorphisms  
associated with severity of β-thalassemia and  
sickle cell disease

Gene	SNP	Effect on HbF level
HBG2	rs7482144 [C>T] = XmnI +/-	+
BCL11A	rs1427407 [C>T]	+
	rs10189857 [A>G]	+
HBS1L-MYB	rs28384513 [A>C]	-
	rs9399137 [T>C]	+

β-Thal  
Modifier  
StripAssay®

4-170

5 mutations covering  
20-50%  
of common variations  
in HbF levels

The Assay

## The ViennaLab Thalassemia StripAssays® meet customer requirements

Requirement	ViennaLab's offer
Easy	Three simple steps. 6 h. Done.
Reliable	Can be automated. Probes for mutations and controls combined on one teststrip.
Versatile	Effective genotyping of DNA from various sample types.
Affordable	Reagents. Thermocycler. Incubator. That is all you need. Software for interpretation of results is optional.

The ViennaLab  $\alpha$ - /  $\beta$ -Globin and  $\beta$ -Thal Modifier StripAssays® combine all these requirements.

### The ViennaLab Thalassemia StripAssays®

- are based on reverse-hybridization of biotinylated PCR products
- combine probes for mutations and controls in a parallel array of allele-specific oligonucleotides
- work with immobilized oligos on a teststrip
- show mutations by enzymatic color reaction easily visible to the naked eye

### The three steps of the ViennaLab Thalassemia StripAssays®

Step	Requirement
<b>1. Amplification:</b> Multiplex PCR-amplification. Simultaneous biotin-labeling	Thermocycler
<b>2. Hybridization:</b> Directly on the StripAssay® teststrips	Incubator
<b>3. Identification:</b> Labeled products detected by streptavidin-alkaline phosphatase	Naked eye or scanner & software

Cat.nos.:  
 $\alpha$ -Globin StripAssay®: 4-160 (10 tests/kit)

$\beta$ -Globin StripAssay® MED: 4-130 (20 tests/kit)  
 $\beta$ -Globin StripAssay® IME: 4-140 (20 tests/kit)  
 $\beta$ -Globin StripAssay® SEA: 4-150 (20 tests/kit)

$\beta$ -Thal Modifier StripAssay®: 4-170 (20 tests/kit)

ViennaLab offers StripAssays® for a wide range of diagnostic applications.  
Visit [www.viennalab.com](http://www.viennalab.com)

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