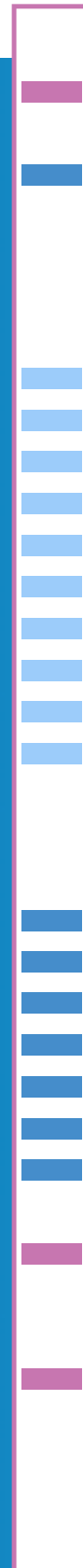


α -Thalassemia

α -Globin StripAssay

In healthy adults 97-98% of total hemoglobin (Hb) is HbA, consisting of two α -globin and two β -globin polypeptides ($\alpha_2\beta_2$). The α -globin chains are encoded by two highly homologous genes, α_1 and α_2 , which are both embedded in tandem in the α -globin gene cluster on chromosome 16. Reduced or absent α -globin synthesis, mainly caused by deletions of one or both α -globin genes and less frequently by point mutations, leads to α -thalassemia (α -thal). The severity of the α -thal phenotype depends on the number of affected genes and the resulting imbalance between α - and β -globin chains. The loss of one of the two α -globin alleles ($-\alpha$) causes α^+ -thal, whereas α^0 -thal is due to inactivation of both α -globin alleles ($--$) within a chromosome. Four intact α -globin genes ($\alpha\alpha/\alpha\alpha$) are present in the diploid genome of a healthy human. Individuals, who inherit only two or three functional genes, have mild anemia and microcytosis. Hb H disease affects subjects with only a single active α -globin gene and presents with moderate to severe hemolytic anemia. The most severe manifestation, the loss of all four α -globin genes ($--/--$), causes homozygous α^0 -thal (Hb Bart's hydrops fetalis syndrome), which is generally associated with death *in utero*. All these various disorders (hemoglobinopathies) are a major public health problem, particularly in Mediterranean countries, the Middle East, India, Asia 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 α -globin alleles are prevalent in each at-risk population, large-scale screening programs are feasible, but require simple and automated test procedures.



α -Globin StripAssay:

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

α -Globin StripAssay:

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

The α -Globin StripAssay provides ready-to-use reagents for 10 tests. The entire assay can be accomplished in less than 6 hours, and may be carried out manually or largely automated.

Principle of the assay:

The α -Globin StripAssay is based on reverse-hybridization of biotinylated PCR products to a parallel array of allele-specific oligonucleotides immobilized on membrane teststrips. The StripAssay provides ready-to-use reagents for completion in three easy steps^{*)}:

- Three-tube multiplex PCR for the specific amplification of the entire $\alpha 1$ and $\alpha 2$ gene, including gap-PCR for single/double gene deletions and gene triplication.
- Hybridization of biotinylated amplification products to oligonucleotide probes on two separate teststrips^{**)}.
- Detection of specifically bound mutant and wild-type alleles by visible enzymatic color reaction.

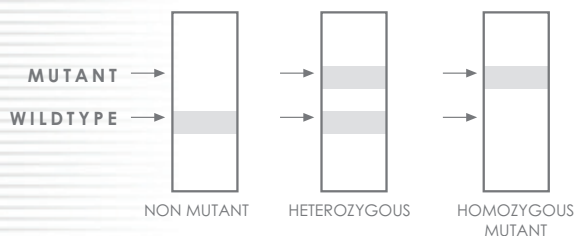
^{*)} In addition, ViennaLab offers reagents for the rapid isolation of genomic DNA from body samples (blood, amniotic fluid, chorionic villous samples). The use of the Spin Micro DNA Extraction Kit with the α -Globin StripAssay is recommended.

^{**)} For genotyping the results of corresponding teststrips A and B are to be combined.

Interpretation of results:

For each point mutation or small deletion, one of three possible staining patterns may be obtained:

1. wild-type probe positive: normal genotype
2. wild-type and mutant probe positive: heterozygous genotype (carrier individual)
3. mutant probe positive: homozygous mutant genotype (affected individual)



The homozygous presence of single/double gene deletions, or their coexistence with point mutations and small deletions, will cause more complex staining patterns, generally defined by the absence of more than one wild-type probe (for details see kit insert). The assay cannot distinguish between heterozygous and homozygous anti-3.7 gene triplication.

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

Position	Sequence alteration	Test strip
$-\alpha^{3.7}$	single gene deletion	A
$-\alpha^{4.2}$	single gene deletion	A
$-(\alpha)^{20.5}$	double gene deletion	A
--MED	double gene deletion	A
--SEA	double gene deletion	A
--THAI	double gene deletion	A
--FIL	double gene deletion	A
$\alpha 1$ cd 14	G>A	A
$\alpha 1$ cd 59	G>A (Hb Adana)	A
$\alpha\alpha$ anti-3.7	gene triplication	B
$\alpha 2$ init. cd	ATG>ACG	B
$\alpha 2$ cd 19	-G	B
$\alpha 2$ IVS1	5bp deletion	B
$\alpha 2$ cd 59	G>A	B
$\alpha 2$ cd 125	T>C (Hb Quong Sze)	B
$\alpha 2$ cd 142	T>C (Hb Constant Spring)	B
$\alpha 2$ cd 142	T>A (Hb Icaria)	B
$\alpha 2$ cd 142	A>T (Hb Pakse)	B
$\alpha 2$ cd 142	A>C (Hb Koya Dora)	B
$\alpha 2$ polyA-1	AATAAA>AATAAG (Saudi type)	B
$\alpha 2$ polyA-2	AATAAA>AATGAA (Turkish type)	B

α -Globin Strip Assay

Cat.no.: 4-160

Spin Micro DNA Extraction Kit

Cat.no.: 2-020

Further StripAssays are available or under development for:

β -Globin (thalassemia), Cardiovascular Disease (CVD), Familial Mediterranean Fever (FMF), Gaucher Disease, Haemochromatosis, Sugar Intolerance (lactose, fructose), Pharmacogenetics, Cancer.



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