

Instructions for Use

SALSA® MLPA® Probemix P102 HBB



See also the MLPA General Protocol, the product descriptions of the SALSA® MLPA® Reagent Kit, SALSA® Binning DNA SD067, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P102 HBB product page on our website to find Certificates of Analysis and a list of related products.

Product Name	SALSA® MLPA® Probemix P102 HBB
Version	D1
Catalogue numbers	P102-025R (25 reactions) P102-050R (50 reactions) P102-100R (100 reactions)
Basic UDI-DI	872021148P1025D
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	Catalogue Numbers
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® Binning DNA SD067	SD067

Storage and Shelf Life

Recommended conditions		
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A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE  2797 ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More Information:	
www.mrcholland.com	
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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version C1, two target probes have been removed and three new target probes have been included. Five probes have been redesigned on the reverse strand, detecting the same ligation site. The length of four probes has been changed. Two reference probes have been replaced and one reference probe has been removed.

1. Intended Purpose

The SALSA MLPA Probemix P102 HBB is an in vitro diagnostic (IVD)¹ or research use only (RUO) semi-quantitative manual assay² for the detection of deletions in the beta-globin (*HBB*) gene cluster and its regulatory region located on chromosome 11p15.4 and the HbS mutation (c.20A>T; p.Glu6Val) in the *HBB* gene in genomic DNA isolated from human peripheral whole blood specimens. In all probands, P102 HBB is intended to confirm a potential cause for beta-thalassaemia. In probands under 12 months, P102 HBB can also be used to confirm a clinical diagnosis of beta-thalassaemia. P102 HBB can also be used to confirm a potential cause for deletional hereditary persistence of foetal haemoglobin (HPFH) caused by deletions in the *HBB* and *HBD* regions including deletions of the *HBB* promoter. The assay can be used for molecular genetic testing of at-risk family members and for carrier screening - identification of individuals with heterozygous variants - for any of the above-mentioned diseases. In addition, this probemix can be used as confirmation of sequencing results for the presence of the HbS mutation c.20A>T; p.Glu6Val in *HBB* causing sickle cell disease (SCD).

Copy number variations (CNVs) detected with P102 HBB should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *HBB* gene region are point mutations, none of which, except for the HbS mutation (c.20A>T; p.Glu6Val) will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, or for the detection of, or screening for, acquired or somatic genetic aberrations.

¹ Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

² To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, treated with RNase (see Appendix) , dissolved in 5 µl TE _{0.1} buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤ 0.10 standard deviation for all probes except for mutation-specific probe. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of beta-thalassaemia, HPFH, or SCD.	
No-DNA Control (Preferably)	• Provided by user • TE_{0.1} buffer instead of DNA • To check for DNA contamination	
Binning DNA (Initial Experiment)	• SALSA Binning DNA SD067, provided by MRC Holland • Recommended in initial experiment to determine suitable bin set • Should never be used as a reference sample	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.
Validation Samples (Required)	• This probemix contains probes that target sequences with natural variation. The validation experiments of this probemix should result in a standard deviation ≤ 0.10 for all reference probes. • In the validation experiments of this probemix, in the majority of samples from healthy individuals, the peak of the mutation-specific probe is expected to be <10% of median peak height of the reference probes while the peak of the wildtype probe should be indicative of a normal copy number.	

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the [MLPA General Protocol](#).

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	Function
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

[Coffalyser.Net](#) should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the [Coffalyser.Net Reference Manual](#) for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our [support portal](#).

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤ 0.10 .

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*HBB* gene cluster)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	2	Normal
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

Typical Results of Probes Targeting Four Copies (136 and 180 nt probes detect a sequence that is present in both *HBG1* and *HBG2*)

Final Ratio (FR)	Copy Number	Description*
0	0	Homozygous deletion of <i>HBG1</i> and <i>HBG2</i>
~0.25	1	Heterozygous deletion of <i>HBG1</i> and homozygous deletion of <i>HBG2</i> or vice versa
~0.50	2	Heterozygous deletion of <i>HBG1</i> and <i>HBG2</i> , or homozygous deletion of <i>HBG1</i> or <i>HBG2</i>
~0.75	3	Heterozygous deletion of <i>HBG1</i> or <i>HBG2</i>
~1.00	4	Normal
~1.25	5	Heterozygous duplication of <i>HBG1</i> or <i>HBG2</i>
~1.50	6	Heterozygous duplication of <i>HBG1</i> and <i>HBG2</i> , or homozygous duplication of <i>HBG1</i> or <i>HBG2</i>
~1.75	7	Heterozygous duplication of <i>HBG1</i> and homozygous duplication of <i>HBG2</i> or vice versa
~2.00	8	Homozygous duplication of <i>HBG1</i> and <i>HBG2</i>

*Please note that other interpretations are possible in some cases. The 136 and 180 nt probes should be interpreted taking into account neighbouring probes.

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

Possible Results of Mutation and Wild Type-Specific Probes

214 nt HbS mutation-specific probe	
Signal Strength	Mutation Status
$\geq 10\%$ median peak height reference probes	HbS mutation is detected (expected only in positive samples)
$< 10\%$ median peak height reference probes	HbS mutation is not detected (expected in most samples from healthy individuals)
220 nt HbS wildtype-specific probe	
Final Ratio (FR)	Mutation Status
~0.5	Heterozygous HbS mutation is detected (expected only in positive samples)
0	Homozygous HbS mutation is detected OR there is a deletion or other mutation near the probe's ligation site (expected only in positive samples)
0.80-1.20	HbS mutation is not detected (expected in most samples from healthy individuals)

Examples of MLPA results obtained with this probemix can be found on the P102 HBB Product Page on www.mrcholland.com.

6. Performance Characteristics

Study	Description																																
Expected values for copy number in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤ 0.10 , the ranges stated in the copy number table in section 5 can be used. Cut-off values for copy number determination were verified with P102 HBB in 87 samples from healthy individuals with normal copy number and four samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.																																
Expected values for point mutation detection in normal and affected populations	The mutation-specific probe will only generate a signal when the HbS mutation is present. The wildtype-specific probe will produce a signal indicating whether the HbS mutation is present in a heterozygous or homozygous state, or absent. A clear signal for the mutation-specific probe (at least 10% of the median peak height of all reference probes in that sample) indicates that the mutation is present. It is not possible to determine the copy number of mutation-specific probes. The expected value for mutation-specific probes was verified with P102 using two mutation positive samples and the expected mutation status was found in all samples tested.																																
Limit of Detection	A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P102 HBB on two samples with known CNVs/mutations status and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA.																																
Interfering substances	SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and haemoglobin) can affect the MLPA reaction. A study using P102 HBB was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs/mutation status. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below. <table><tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr><tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td><u>Copy number</u>: Expected FR for 189/189 measurements <u>Mutation and wildtype</u>: Expected mutation status for 18/18 measurements</td></tr><tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td><u>Copy number</u>: Expected FR for 189/189 measurements <u>Mutation and wildtype</u>: Expected mutation status for 18/18 measurements</td></tr><tr><td>Fe³⁺ (FeCl₃)</td><td>Exogenous – DNA extraction</td><td>1 μM</td><td><u>Copy number</u>: Expected FR for 189/189 measurements <u>Mutation and wildtype</u>: Expected mutation status for 18/18 measurements</td></tr><tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td><u>Copy number</u>: Expected FR for 189/189 measurements <u>Mutation and wildtype</u>: Expected mutation status for 18/18 measurements</td></tr><tr><td>Haemoglobin</td><td>Endogenous – blood sample</td><td>0.02 μg/μl</td><td><u>Copy number</u>: Expected FR for 185/189 measurements <u>Mutation and wildtype</u>: Expected mutation status for 18/18 measurements</td></tr><tr><td>RNA</td><td>Endogenous – blood sample</td><td>0.8 ng/μl</td><td><u>Copy number</u>: Expected FR for 129/189 measurements <u>Mutation and wildtype</u>: Expected mutation status for 2/18 measurements</td></tr><tr><td>Bilirubin</td><td>Endogenous – blood sample (newborn)</td><td>140 mg/L</td><td><u>Copy number</u>: Expected FR for 189/189 measurements <u>Mutation and wildtype</u>: Expected mutation status for 18/18 measurements</td></tr></table> * Results are summarised for all probes across all three samples tested. RNA was the substance which had the greatest effect on both copy number and mutation status determination. Incorrect results were obtained in all samples. Since HBB is heavily expressed in red blood cells, an RNase treatment of samples is essential for (whole-)blood-derived samples. Without RNase treatment, HBB mRNA can bind to probes that detect a sequence within the HBB exons, thereby reducing the effective concentration of probes. Haemoglobin had an effect on copy number determination only in the wildtype sample: four ambiguous measurements were obtained. Importantly, warnings were obtained for this sample using Coffalyser.Net software. DNA extraction methods from blood remove haemoglobin and during testing of 23 samples	Interferent	Source	Testing Concentration	Results*	EDTA	Exogenous – specimen collection tubes	1.5 mM	<u>Copy number</u> : Expected FR for 189/189 measurements <u>Mutation and wildtype</u> : Expected mutation status for 18/18 measurements	NaCl	Exogenous – DNA extraction	40 mM	<u>Copy number</u> : Expected FR for 189/189 measurements <u>Mutation and wildtype</u> : Expected mutation status for 18/18 measurements	Fe ³⁺ (FeCl ₃)	Exogenous – DNA extraction	1 μ M	<u>Copy number</u> : Expected FR for 189/189 measurements <u>Mutation and wildtype</u> : Expected mutation status for 18/18 measurements	Heparin	Exogenous – specimen collection tubes	0.02 U/mL	<u>Copy number</u> : Expected FR for 189/189 measurements <u>Mutation and wildtype</u> : Expected mutation status for 18/18 measurements	Haemoglobin	Endogenous – blood sample	0.02 μ g/ μ l	<u>Copy number</u> : Expected FR for 185/189 measurements <u>Mutation and wildtype</u> : Expected mutation status for 18/18 measurements	RNA	Endogenous – blood sample	0.8 ng/ μ l	<u>Copy number</u> : Expected FR for 129/189 measurements <u>Mutation and wildtype</u> : Expected mutation status for 2/18 measurements	Bilirubin	Endogenous – blood sample (newborn)	140 mg/L	<u>Copy number</u> : Expected FR for 189/189 measurements <u>Mutation and wildtype</u> : Expected mutation status for 18/18 measurements
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Study	Description
	extracted from blood the expected final ratios were found. Therefore, it is only when haemoglobin is in excess that deviating probe signals can be found. None of the other tested substances were shown to have an effect on P102 HBB. To minimise variability across samples, all samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible.
Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, four previously genotyped samples were tested using P102 HBB and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number/mutation status over multiple days, and by multiple operators. Results showed a correct call in 882/882 measurements for copy number and 84/84 correct calls for mutation status data points, leading to a precision of >99%.
Clinical validity*	β-thalassemia: Less than 1% of β-thalassemia cases are explained by deletions in the <i>HBB</i> gene cluster (GeneReviews) SCD: 100% of SCD cases are caused by the presence of at least one haemoglobin S allele (HbS; c.20A>T; p.Glu6Val in <i>HBB</i>) and a second <i>HBB</i> pathogenic variant resulting in abnormal haemoglobin polymerization (GeneReviews) Deletional HPFH: 100% of deletional HPFH cases are caused by large deletions in the β-globin gene cluster effecting both <i>HBD</i> and <i>HBB</i>, specifically involving the <i>HBB</i> promoter (Thein and Wood 2009) *(Based on a 2008-2025 literature review)

Summary of Safety and Performance (SSP)

The SSP is available in the European database on medical devices (Eudamed), <https://ec.europa.eu/tools/eudamed>, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Mutation	Length (nt)	Probe number	Warnings
11p15.4	TRIM68		504.3 kb		160	18244-L22953	~
11p15.4	OR52A5		45.3 kb		227	18246-L30180	~
11p15.4	OR52A1		48.3 kb		355	18251-L27003	~
11p15.4	OR51V1		15.8 kb		486	18253-L22962	~
11p15.4	HBB	Downstream	9.1 kb		274	11980-L12803	~ Ø
11p15.4	HBB	Downstream	0.3 kb		173	05836-L06321	Ø
11p15.4	HBB	Downstream	0.3 kb		208	11885-L25666	Ø
11p15.4	HBB	Exon 3	0.9 kb		166	13619-L15073	°
11p15.4	HBB	Intron 2	0.4 kb		196	05833-L05335	Ø
11p15.4	HBB	Exon 1	0.1 kb		154	11883-L12683	+
11p15.4	HBB	Exon 1	0.1 kb	c.20A>T	214	21234-L29609	§ °
11p15.4	HBB	Exon 1		c.20A>T (wildtype)	220	21234-L29610	§ ° ∞
11p15.4	HBB	Exon 1	0.1 kb		189	05828-L05332	°
11p15.4	HBB	Upstream	0.8 kb		148	05827-L06319	Ø
11p15.4	HBB	Upstream	1.9 kb		365	11982-L24242	Ø
11p15.4	HBB	Upstream	3.0 kb		337	05824-L05328	Ø
11p15.4	HBD	Exon 3	0.2 kb		391	18108-SP0126-L15076	Ж
11p15.4	HBD	Exon 3	1.5 kb		346	21235-L29611	° #
11p15.4	HBD	Exon 1	3.3 kb		312	21555-L30397	+
11p15.4	HBD	Upstream	4.1 kb		420	05821-L06327	Ø
11p15.4	HBBP1	Exon 3	1.5 kb		382	21239-L29615	
11p15.4	HBBP1	Exon 1	2.1 kb		445	06400-L05323	°
11p15.4	HBBP1	Upstream	2.8 kb		283	18248-SP0631-L27002	Ж Ø
11p15.4	HBG1	Exon 3	1.1 kb		436	21237-L29613	° ∫
11p15.4	HBG1 & HBG2	Exon 2 HBG1	0.2 kb		136	21231-L29607	° »
11p15.4	HBG1 & HBG2	Exon 1 HBG1	1.4 kb		180	21230-L30179	° »
11p15.4	HBG1	Upstream	1.9 kb		409	05817-L05321	Ø
11p15.4	HBG2	Exon 3	3.0 kb		472	18109-SP0127-L27005	Ж +
11p15.4	HBG2	Exon 3	1.2 kb		427	21237-L29612	° ∫
11p15.4	HBG1 & HBG2	Exon 2 HBG2	0.2 kb		136	21231-L29607	° »
11p15.4	HBG1 & HBG2	Exon 1 HBG2	1.4 kb		180	21230-L30179	° »
11p15.4	HBG2	Upstream	13.8 kb		373	05815-L05319	Ø
11p15.4	HBE1	Exon 1	6.0 kb		463	05813-L05317	
11p15.4	HBB-HS1 Region	HS1	4.8 kb		240	12189-L30182	
11p15.4	HBB-HS2 Region	HS2	4.1 kb		292	06395-L05315	
11p15.4	HBB-HS3 Region	HS3	1.1 kb		233	21238-L30181	
11p15.4	HBB-HS3 Region	HS3	2.1 kb		330	21240-L29616	
11p15.4	HBB-HS4 Region	HS4	0.4 kb		268	05807-L22540	
11p15.4	HBB-HS4 Region	HS4	6.8 kb		304	05806-L22542	
11p15.4	HBB-HS5 Region	HS5 Upstream	28.9 kb		261	05804-L27001	Ø
11p15.4	OR51B2		66.1 kb		298	18249-L24239	~
11p15.4	OR51M1				253	18247-SP0630-L27000	Ж ~
1p	Reference				142	18900-L24495	
3q	Reference				130	16316-L18705	
8q	Reference				202	18560-L24870	
12q	Reference				319	14404-L16086	
13q	Reference				494	04274-L25060	
14q	Reference				401	20870-L28888	
15q	Reference				245	11213-L14694	
17q	Reference				454	04075-L03310	
21q	Reference				502	18161-L25061	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

The HBB, HBD, HBBP1, HBG1, HBG2, and HBB-HSx region exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

- § The 214 nt probe will only give a signal when the sickle cell-specific point mutation (rs334) is present. The 220 nt probe detects the wildtype sequence at the location of this mutation. **Warning: Signal of the 220 nt probe is reduced ~50% by the HbS mutation. This signal is reduced ~25-50% by other mutations near the ligation site (e.g. the haemoglobin C mutation (*HBB:c.19G>A*), *c.25_26delAA* and *c.17_18delCT*).**
 - ° This probe is expected to be more variable in samples that contain RNA (see Appendix). RNase treatment of DNA samples is recommended for this product.
 - ∞ Wild type sequence detected. **Warning: Signal of the 220 nt probe is reduced ~50% by the HbS mutation. This signal is reduced ~25-50% by other mutations near the ligation site (e.g. the haemoglobin C mutation (*HBB:c.19G>A*), *c.25_26delAA* and *c.17_18delCT*).** A positive result must be confirmed by another method.
 - These probes are flanking probes, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probes are unlikely to be related to the condition tested.
 - ✕ These probes consist of three parts and have two ligation sites. A low signal of these probes can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
 - ∅ These probes target sequences outside of the known coding region. Copy number alterations of only one of these probes are of unknown clinical significance.
 - » These probes detect sequences that are present in both *HBG1* and *HBG2*. Deletion/duplication of a single target site results in a 20-25% decrease/increase in signal intensity of these probes.
 - # The specificity of this probe relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
 - + The ligation site(s) of these probes is/are >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.
 - ∫ Please disregard apparent copy number changes detected only by the 427 nt and 436 nt *HBG1* & *HBG2* probes. The sequences detected by these probes have only a single nucleotide difference. Due to their close proximity (5 kb), it is likely that the *HBG2* sequence at this position is changed in some healthy individuals due to gene conversion in a *HBG1* sequence (SNV rs200060381) and vice versa (SNV rs56205611), without any consequences. A single (benign) nucleotide change in the sample DNA can generate an apparent deletion detected by the 427 nt probe and a duplication detected by the 436 nt probe or vice versa.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product:** none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. Large deletions are often detected with this probemix. The slope correction algorithm in Coffalyser.Net may confuse a large deletion for sloping, leading to an incorrectly applied slope correction and a false warning or ambiguous results for multiple probes. Contact MRC Holland if you suspect that this happened.
6. The presence of a clear signal (at least 10% of the median peak height of all reference probes in the sample) for the 214 nt probe, indicates the presence of the HbS mutation (*HBB:c.20A>T*, rs334). This probe will not generate a signal in the majority of samples. The 220 nt probe indicates the copy number of the wildtype allele at this location. HbS is the most frequent pathological haemoglobin variant worldwide and is predominantly found in Africans and African-Americans. Homozygosity for this mutation or compound heterozygosity (HbS and beta-thalassaemia) leads to sickle-cell disease (Weatherall 2010).
7. Results of the 214 nt and 220 nt probes must be interpreted with caution. Unreliable results may be obtained due to presence of a mutation near the ligation site (e.g. the haemoglobin C mutation (*HBB:c.19G>A*), *c.25_26delAA* and *c.17_18delCT*), which might influence hybridisation of the probes to the target sequence and/or ligation of the probes. It is, therefore, strongly recommended to use the P102 HBB probemix in combination with sequence analysis of the *HBB* gene. Results of the sequence analysis should always be leading; results obtained with the 214 nt and 220 nt probes should be used as confirmation of sequencing results.
8. Rare cases of mosaic segmental uniparental isodisomy (UPID) of chromosome 11p have been reported. If a somatic mutation that causes UPID occurs in the haematopoietic tissue, the number of cells that become homozygous for the mutated allele may outgrow the heterozygous haematopoietic cells during life (loss of heterozygosity, LOH). LOH may also be a consequence of acquired large deletions in the same region. This may lead to late-onset transfusion-dependent beta-thalassaemia. If consecutive probes show the same ambiguous ratio (for example, 0.7), presence of UPID should be considered (Badens et al. 2002; Chang et al. 2008; Bento et al. 2013; Hartevelde et al. 2013).
9. The different globin genes in this locus are being expressed at different stages of development. Therefore, phenotype is not only dependent on which gene is affected, but also on stage of development. For example, a deletion involving the *HBE1*, *HBG1*, *HBG2*, *HBD* and *HBB* genes can lead to severe haemolytic anaemia during embryonic and foetal development which may require intra-uterine blood transfusion. During adult life, this type of deletion causes mild microcytic hypochromic anaemia, comparable to betathalassaemia carriers (Hartevelde et al. 2003; Game et al. 2003; Shalev et al. 2013).
10. The human globin genes have evolved from a single ancestor gene; therefore, all globin genes have a similar structure. The *HBG1* and *HBG2* genes are almost identical, differing by only a few nucleotides. These genes are, therefore, prone to homologous recombination, leading to either deletions, duplications, gene conversions, or

formation of hybrid genes, most of which can be detected by the P102 HBB probemix. Deletions or duplications of the *HBG1* and *HBG2* globin genes must be interpreted with caution; the majority of these rearrangements are not clinically relevant (Neumann et al. 2010).

11. The nuclease hypersensitive site sequences HS1, HS2, HS3, HS4 and HS5 constitute the locus control region (LCR) of the beta-globin gene cluster. This LCR directs correct expression of the globin genes during the different stages of development. A mutation or deletion in the LCR disrupts its function, which leads to a thalassaemia phenotype although the globin genes themselves are present and intact (Stamatoyannopoulos 2005).

Technique-specific precautions
See the [MLPA General Protocol](#).

8. Limitations

Technique-specific limitations
See the [MLPA General Protocol](#).

9. References Cited in this IFU

1. Badens C et al. (2002). A novel mechanism for thalassaemia intermedia. *Lancet*. 359: 132-3.
2. Bento C et al. (2013). β Thalassaemia major due to acquired uniparental disomy in a previously healthy adolescent. *Haematologica*. 98(1):e4-6.
3. Chang JG et al. (2008). Beta-thalassemia major evolution from beta-thalassemia minor is associated with paternal uniparental isodisomy of chromosome 11p15. *Haematologica*. 93(6):913-6.
4. Game L et al. (2003). A novel deletion causing (epsilon gamma delta beta) degrees thalassaemia in a Chilean family. *Br J Haematol*. 123(1):154-9.
5. Hartevelde CL et al. (2003). Novel 112 kb (epsilon gamma delta beta) degrees thalassaemia deletion in a Dutch family. *Br J Haematol*. 122(5):855-8.
6. Hartevelde CL et al. (2013). Mosaic segmental uniparental isodisomy and progressive clonal selection: a common mechanism of late onset beta-thalassemia major. *Haematologica*. 98(5):691-5.
7. Neumann R et al. (2010). Dynamics and processes of copy number instability in human γ -globin genes. *Proc Natl Acad Sci USA*. 107(18): 8304-9.
8. Shalev H et al. (2013). A novel epsilon gamma delta beta thalassaemia presenting with pregnancy complications and severe neonatal anemia. *Eur J Haematol*. 90(2):127-33.
9. Stamatoyannopoulos G. (2005). Control of globin gene expression during development and erythroid differentiation. *Exp Hematol* 33 (3):259-71.
10. Thein, S. L. and W. G. Wood (2009). The Molecular Basis of β Thalassaemia, $\delta\beta$ Thalassaemia, and Hereditary Persistence of Fetal Hemoglobin. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. M. H. Steinberg, B. G. Forget, D. R. Higgs and D. J. Weatherall. Cambridge, Cambridge University Press: 323-356.
11. Weatherall DJ (2010). The inherited diseases of hemoglobin are an emerging global health burden. *Blood* 115(22): 4331-6.

Implemented changes in the product description

Version D1-06 – 04 May 2026 (03S)

- Basic UDI-DI added.
- Intended purpose updated: the word manual has been added; only deletions in *HBB* gene cluster and its regulatory region are relevant; specifications with regards to which specific mutation the term "HbS" refers to have been added; the diagnosis of β -thalassaemia is different for probands under and over 1 year of age, specifications with regards to the function of the assay have therefore been made in relation to this; the specification of the use of this assay for carrier detection (in β -thalassaemia and deletional HPFH) has been made; the subtype of HPFH that P102 HBB can detect has been specified, and the related regions added; SCA now falls under the term SCD, so SCA has been removed; the contra-indication for population screening has been removed.
- Performance characteristics updated with data from analytical performance experiments, and accompanying reference added.
- Reference to the SSP has been added.
- Exon numbering (in parenthesis) from version D1-04 has been removed from the probe content table, along with the accompanying footnote.
- The distance between probe 21237-L29613 and probe 21231-L29607 was added in the probe content table.
- Probemix-specific limitation concerning the determination of zygosity with the mutation-specific probe removed (as a wildtype probe is present).
- Minor textual clarifications were carried out.
- Probemix is now IVDR certified.

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10. Appendix

RNase sample treatment (essential for HBA and HBB MLPA probemixes)

Since *HBB* is heavily expressed in red blood cells, an RNase treatment of samples is essential for (whole-)blood-derived samples*. Without RNase treatment, *HBB* mRNA can compete with or bind to probes that detect a sequence within the *HBB* exons, thereby reducing the effective concentration of probes. Please note that some automatic DNA purification methods (e.g. Roche MagNA Pure) do not include an RNase treatment. The following method can be used to treat RNA containing DNA samples:

Mix 4 µl sample and 1 µl 0.5 mg/ml RNase A. Incubate 30 minutes at 37°C. Continue with the 5 minutes 98°C DNA denaturation step of the MLPA General Protocol.

When using between 50-250 ng of DNA, the DNA concentration after the addition of RNase A should be 10-50 ng/µl.

RNase A is extremely stable; it can be diluted in TE and stored at -20°C. We recommend RNase A from Promega (A7973; 4 mg/ml solution), diluted 8 fold in TE (1 ml of 4 mg/ml RNase is sufficient for ~8000 samples). Do not use more than the recommended amount.

**Please note that RNase treatment is not necessary for SALSA Binning DNA SD067.*