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	n.a.
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		

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 CE ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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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) semiquantitative assay[2] for the detection of deletions or duplications in the beta-globin (*HBB*) gene cluster and its regulatory region located on chromosome 11p15.4 in genomic DNA isolated from human peripheral whole blood specimens. P102 HBB is intended to confirm a potential cause for and clinical diagnosis of beta-thalassaemia or hereditary persistence of foetal haemoglobin (HPFH) and for molecular genetic testing of at-risk family members. In addition, this probemix can be used as confirmation of sequencing results for the presence of the HbS mutation causing sickle cell anaemia (SCA) or 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, most of which will not 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, population screening, 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, SALSA Binning DNA SD067, and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, **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 probes. • 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, SCA, 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 • Required 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 target 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, the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.	

*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
FR = 0	0	Homozygous deletion
FR ≈ 0.25	1	Heterozygous deletion of *HBG1* and homozygous deletion of *HBG2* or *vice versa*
FR ≈ 0.50	2	Heterozygous deletion of *HBG1* and *HBG2*, or homozygous deletion of *HBG1* or *HBG2*
FR ≈ 0.75	3	Heterozygous deletion of *HBG1* or *HBG2*
FR ≈ 1.00	**4**	Normal
FR ≈ 1.25	5	Heterozygous duplication of *HBG1* or *HBG2*
FR ≈ 1.50	6	Heterozygous duplication of *HBG1* and *HBG2*, or homozygous duplication of *HBG1* or *HBG2*
FR ≈ 1.75	7	Heterozygous duplication of *HBG1* and homozygous duplication of *HBG2* or *vice versa*
FR ≈ 2.00	8	Homozygous duplication of *HBG1* and *HBG2*

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**
≥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

Most of the beta-thalassaemia mutations are caused by point mutations in *HBB* and should be identified by sequencing analysis. Approximately 10% of cases are caused by deletions of (a part of) the *HBB* gene or *HBB* gene cluster (http://www.ncbi.nlm.nih.gov/books/NBK1426/). Screening for deletions in the *HBB* gene cluster by MLPA is recommended for beta-thalassaemia patients in whom sequence analysis of the beta-globin gene is negative. SCD and SCA are caused by the *HBB*:c.20A>T mutation (HbS mutation) and all cases can be identified by sequencing analysis of the *HBB* gene (https://www.ncbi.nlm.nih.gov/books/NBK1377/). The P102 HBB probemix can be used to confirm presence of the HbS mutation. The addition of MLPA to a diagnostic routine for beta-thalassaemia and HPFH leads to a definitive diagnosis in nearly all patients presenting with a beta-thalassaemia or HPFH phenotype. The analytical sensitivity and specificity for the detection of the HbS mutation and copy number changes in the beta-globin gene cluster (based on a 2008-2024 literature review) is very high and can be considered >99%.

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 (Intron 1)	0.1 kb		154	11883-L12683	+
11p15.4	*HBB*	Exon 1		c.20A>T	214	21234-L29609	§ °
11p15.4	*HBB*	Exon 1	0.1 kb	c.20A>T	220	21234-L29610	§ ° ∞
11p15.4	*HBB*	Exon 1	0.1 kb		189	05828-L05332	°
11p15.4	*HBB*	Upstream (Exon 1)	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			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. Annotations of several probes with targets at the edge of or slightly outside the coding region, were altered. The exon numbering from the previous version of this Product Description is disclosed between brackets when a discrepancy is present.
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.
Ж	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.
Ø	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 (17 nt after the stop codon), 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 (SNP rs200060381) and vice versa (SNP 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 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 haemotopoietic 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; Harteveld 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 (Harteveld 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 probemix. Deletions or duplications of the *HBG1* and *HBG2* globin genes must be interpreted with caution; the majority of these rearrangements is 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

Probemix-specific limitations
1. The mutation-specific probe(s) can only detect the presence of the mutation and should not be used to determine zygosity.

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 Thalassemia major due to acquired uniparental disomy in a previously healthy adolescent. *Haematologica*. 98(1):e4-6.
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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.
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6. Harteveld 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 thalassemia 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. 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 D1-04 – 11 March 2025 (03S)
- Product description updated to new template.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- SNVs rs554807922, rs551352636, rs543925348, rs575180790, rs112330205, rs574030447, rs189836443 can affect the probe signal. However, the warning(s) for this/these SNV(s) present in previous product description versions has/have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Warnings for a ligation site >20nt from the nearest exon were added for probes 18109-SP0127-L27005 and 11883-L12683.
- Warnings for probes targeting sequences outside of the known coding region were added for 11980-L12803, 05836-L06321, 11885-L25666, 05833-L05335, 05827-L06319, 11982-L24242, 05824-L05328, 18248-SP0631-L27002, 05817-L05321, 05815-L05319, and 18247-SP0630-L27000.
- Warning for probes detecting sequences that are present in both *HBG1* and *HBG2* added for 21231-L29607 and 21230-L30179.
- Final DNA concentration after the addition of RNase A was added to the RNase sample treatment box in the Appendix.
- Interpretation-aid tables present in the previous version of this Product Description will now be downloadable from the P102 HBB Product Page.

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 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.