MRC Holland SALSA® MLPA®

Instructions for Use

SALSA® MLPA® Probemix P093 HHT/HPAH

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P093 HHT/HPAH product page on our website to find Certificates of Analysis and a list of related products.

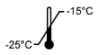
Product Name	**SALSA® MLPA® Probemix P093 HHT/HPAH**
Version	C2
Catalogue numbers	P093-025R (25 reactions) P093-050R (50 reactions) P093-100R (100 reactions)
Basic UDI-DI	872021148P09365
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL
RUO	ALL OTHER COUNTRIES

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

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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.

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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, the length of several probes has been adjusted.

1. Intended Purpose

The SALSA MLPA Probemix P093 HHT/HPAH is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions in the *ACVRL1* gene and deletions or duplications in the *BMPR2* and *ENG* genes in genomic DNA isolated from human peripheral whole blood specimens. P093 HHT/HPAH is intended to confirm a potential cause for or clinical diagnosis of Hereditary Hemorrhagic Telangiectasia (HHT) (deletions in *ACVRL1*, and deletions and duplications in *ENG*), and to confirm a potential cause for and clinical diagnosis of Heritable Pulmonary Arterial Hypertension (HPAH) (deletions and duplications in *BMPR2*). This assay is additionally intended for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P093 HHT/HPAH 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 *ENG*, *ACVRL1* and *BMPR2* genes are point mutations, none of which 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, 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, and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, 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. At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of HHT or HPAH.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
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.

*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 (*ENG*, *BMPR2*, and *ACVRL1*)

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

The tables illustrate the relationship between final 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.

6. Performance Characteristics

Study	Description
Expected values for copy numbers 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 the product description can be used. Cut-off values for copy number determination were verified with P093 HHT/HPAH in 44 samples from healthy individuals with normal copy number and three samples with known CNVs. The expected FRs for the corresponding copy number were found in over 99% of measurements across all tested samples. Less than 1% of measurements yielded FRs in the ambiguous range.
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 P093 HHT/HPAH 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 hemoglobin) can affect the MLPA reaction. A study using SALSA MLPA Probemix P093 HHT/HPAH was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs. 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.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 598/602 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 641/645 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 640/645 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 639/645 measurements
Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 439/645 measurements

* Results are summarised for all 43 target probes across all five samples tested.

	Potential interference with the MLPA reaction was assessed by testing both endogenous (hemoglobin) and exogenous interfering substances (EDTA, heparin, salts (NaCl), and $FeCl_3$) using P093 HHT/HPAH. Hemoglobin had the largest effect on the FRs: all samples yielded either ambiguous results or a combination of ambiguous and incorrect final ratios. DNA extraction methods from blood remove hemoglobin. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found. $FeCl_3$, heparin, EDTA and NaCl exhibited minimal interference with P093 HHT/HPAH assay. In the few instances where interference occurred, the resulting FRs fell within the ambiguous range. Such values are not indicative of false positives and would at most, lead to delayed reporting. 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 samples were tested using P093 HHT/HPAH and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 3174/3225 data points, leading to a precision of >98%.
Clinical validity*	*BMPR2*: 5% - 20% of HPAH is caused by deletions/duplications in *BMPR2* (Aldred et al. 2006, Graf et al. 2018, Machado et al. 2009). *ACVRL1*: 5% - 6% of HHT is caused by deletions in *ACVRL1*[1]. *ENG*: 4% - 5% of HHT is caused by deletions/duplications in *ENG*[1]. *Based on a 2005-2025 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.

[1] Gene Reviews Hereditary Hemorrhagic Telangiectasia, National Library of Medicine (NIH)

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
2q33.1	*BMPR2*	Exon 1	0.5 kb	154	08198-L11404	
2q33.1	*BMPR2*	Exon 1	87.4 kb	137	04004-L03427	
2q33.1	*BMPR2*	Exon 2	2.7 kb	190	04005-L03428	
2q33.1	*BMPR2*	Exon 3	46.2 kb	270	04338-L03966	
2q33.1	*BMPR2*	Exon 4	1.2 kb	328	04007-L03430	
2q33.1	*BMPR2*	Exon 5	4.1 kb	409	04008-L28921	
2q33.1	*BMPR2*	Exon 6	1.2 kb	433	04009-L28924	
2q33.1	*BMPR2*	Exon 7	10.7 kb	148	04010-L03433	
2q33.1	*BMPR2*	Exon 8	1.7 kb	172	04011-L03434	
2q33.1	*BMPR2*	Exon 9	9.8 kb	220	04012-L03435	
2q33.1	*BMPR2*	Exon 10	10.4 kb	304	04013-L20480	
2q33.1	*BMPR2*	Exon 11	2.7 kb	355	04014-L03437	
2q33.1	*BMPR2*	Exon 12	4.4 kb	417	04015-L28922	
2q33.1	*BMPR2*	Exon 13		441	04016-L28925	
9q34.11	*ENG*	Exon 15 (Exon 14b)	0.4 kb	400	03788-L28920	
9q34.11	*ENG*	Exon 15 (Exon 14b)	0.2 kb	472	08502-L28929	
9q34.11	*ENG*	Exon 14 (Exon 14b)	1.2 kb	449	08501-L28926	
9q34.11	*ENG*	Exon 13	1.0 kb	364	03013-L02453	
9q34.11	*ENG*	Exon 12	0.6 kb	481	08500-L28930	
9q34.11	*ENG*	Exon 11	0.8 kb	337	03583-L03013	
9q34.11	*ENG*	Exon 10	0.3 kb	376	05237-L28917	
9q34.11	*ENG*	Exon 9	4.5 kb	312	03011-L20481	
9q34.11	*ENG*	Exon 8	0.4 kb	283	07680-L07402	
9q34.11	*ENG*	Exon 7	0.4 kb	393	05236-L28919	
9q34.11	*ENG*	Exon 6	0.6 kb	256	03009-L02449	
9q34.11	*ENG*	Exon 5	0.7 kb	142	05235-L04615	
9q34.11	*ENG*	Exon 4	3.2 kb	232	03008-L05740	
9q34.11	*ENG*	Exon 3	13.4 kb	203	03007-L03614	
9q34.11	*ENG*	Exon 2	0.0 kb	184	03006-L02446	
9q34.11	*ENG*	Exon 2	11.1 kb	457	05234-L28927	
9q34.11	*ENG*	Exon 1	0.8 kb	166	03005-L02445	
9q34.11	*ENG*	Upstream (Exon 1)		465	05233-L28928	Ø
12q13.13	*ACVRL1*	Exon 1	0.0 kb	214	03789-L05739	+
12q13.13	*ACVRL1*	Exon 1	5.0 kb	178	05229-L04609	+
12q13.13	*ACVRL1*	Exon 2	0.8 kb	244	03016-L20477	
12q13.13	*ACVRL1*	Exon 3	0.4 kb	209	05230-L05738	
12q13.13	*ACVRL1*	Exon 4	0.3 kb	265	03017-L02457	
12q13.13	*ACVRL1*	Exon 5	0.4 kb	226	05231-L04611	
12q13.13	*ACVRL1*	Exon 6	0.7 kb	297	03018-L20479	
12q13.13	*ACVRL1*	Exon 7	0.9 kb	276	08196-L11406	
12q13.13	*ACVRL1*	Exon 8	2.9 kb	319	03019-L02459	
12q13.13	*ACVRL1*	Exon 9	1.9 kb	346	03020-L28916	
12q13.13	*ACVRL1*	Exon 10		160	03790-L03965	
1p	Reference			250	02869-L20478	
3q	Reference			238	08858-L08980	
4q	Reference			385	16932-L28918	
5q	Reference			130	00797-L00463	
6p	Reference			197	10688-L11270	
9p	Reference			290	15677-L17643	
17q	Reference			490	09966-L28931	
18q	Reference			426	16449-L28923	

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 *BMPR2, ENG,* and *ACVRL1* 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.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

Ø This probe targets a sequence outside of the known coding region. Copy number alterations of only this probe is of unknown clinical significance.

\+ The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.

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.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. In most populations, the major cause of genetic defects in the *BMPR2*, *ACVRL1* and *ENG* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P093 HHT/HPAH.
2. MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region do exist but remain undetected.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Aldred MA et al. (2006). BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. *Hum Mutat.* 27:212-213.
2. Graf S et al. (2018). Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. *Nat Commun.* 9:1416.
3. Machado RD et al. (2009). Genetics and genomics of pulmonary arterial hypertension. *J Am Coll Cardiol.* 54:S32-S42.

Implemented changes in the product description

Version C2-07 – 08 June 2026 (03S)

- Product Description adapted to a new template.
- Basic UDI-DI added.
- BSI registration number added.
- Intended purpose updated to specify that the assay is manual, the use of P093 HHT/HPAH is intended for detecting deletions in *ACVRL1* (duplications removed), and P093 HHT/HPAH is used for confirming a potential cause for or clinical diagnosis of HHT. Minor textual changes.
- Performance characteristics updated.
- Reference to SSP added.
- Description of target probe at the edge of or slightly outside the coding region has been adjusted. No change in the actual target sites.
- Exon numbering updated for ENG probes 03788-L28920, 08502-L28929, 08501-L28926, and 05233-L28928.
- Probemix is now IVDR-certified.

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