Instructions for Use

SALSA® MLPA® Probemix P158 JPS

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 P158 JPS product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P158 JPS**
Version	**D1**
Catalogue numbers	**P158-025R (25 reactions)** **P158-050R (50 reactions)** **P158-100R (100 reactions)**
Basic UDI-DI	**872021148P15868**
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

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 2797
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 in which the user and/or the patient is located.

Changes in this Product Version:
As compared to version C2, one **BMPR1A** and one **SMAD4** probe have been replaced, one **PTEN** probe has been added, one **SMAD4** flanking probe has been removed, two reference probes have been replaced and one has been added. Also, the length of several probes has been adjusted.

1. Intended Purpose

The SALSA MLPA Probemix P158 JPS is an in vitro diagnostic (IVD)[1] or research use only (RUO) semiquantitative manual assay[2] for the detection of deletions in the *BMPR1A* and *SMAD4* genes, as well as the 10q22-q23 microdeletion, which contains both *BMPR1A* and *PTEN*, in genomic DNA isolated from human peripheral whole blood specimens. P158 JPS is intended to confirm a potential cause for and, in the case of inconclusive clinical features, to confirm a clinical diagnosis of Juvenile Polyposis Syndrome (JPS), and for molecular genetic testing of at-risk family members.

Deletions detected with P158 JPS should be confirmed with a different technique. In particular, deletions detected by only a single probe always require confirmation by another method. Most defects in the *BMPR1A*, *SMAD4* and *PTEN* 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, e.g. from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[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, free from heparin, 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 JPS.	
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 (*SMAD4*, *BMPR1A*, and *PTEN*)

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 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.

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 the product description can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P158 JPS in 65 samples from healthy individuals with normal copy number and 11 samples with known CNVs. For almost all measurements, the expected final ratios (FRs) for the corresponding copy number were found in all samples tested. Only for six positive samples were ambiguous FRs obtained for one or two measurements, which would at most lead to delayed results.
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 SALSA MLPA Probemix P158 JPS on two samples with known CNVs and on one normal sample, and, using both the lower and upper input amount of DNA, expected results were obtained in all but one measurement. The deviating measurement was observed using 50ng of DNA, and led to an ambiguous FR, which would at most lead to delayed results.
Interfering substances	SNPs 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 P158 JPS was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on two samples with known CNVs and one normal sample. 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	Expected FR for 349/351 measurements
NaCl	Exogenous – DNA extraction	40 mM	Expected FR for 351/351 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Expected FR for 349/351 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Expected FR for 350/351 measurements
Haemoglobin	Endogenous – blood sample	0.02 µg/µl	Expected FR for 289/351 measurements

* Results are summarised for BMPR1A, PTEN and SMAD4 probes across all three samples tested.

Study	Description
	NaCl did not interfere with copy number determination, while an effect on the FRs was observed for a low number of probes with EDTA, $FeCl_3$, and heparin. The deviating ratios produced in these cases were ambiguous. This would only lead to delayed results. Haemoglobin had the largest effect on the FRs, as the wrong copy number was determined in several cases. DNA extraction methods from blood remove hemoglobin. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found. Additionally, Coffalyser.Net issues warnings for the samples in which the interferents showed an effect, as well as lowered quality scores. 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-reactions	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, nine previously genotyped samples were tested using SALSA MLPA Probemix P158 JPS and found to have the expected results in 694/702 measurements, leading to a trueness of 99%. 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 1739/1755 data points, leading to a precision of 99%.
Clinical validity*	*BMPR1A:* 15% of JPS is explained by large deletions in *BMPR1A* (Aretz et al. 2007, Calva-Cerqueira et al. 2009, Latchford et al. 2012, van Hattem et al. 2008) *BMPR1A/PTEN* microdeletion*:* 1-3% of JPS is explained by microdeletions encompassing both *BMPR1A* and *PTEN* (Dahdaleh et al. 2012). *SMAD4:* 17% of JPS is explained by large deletions in *SMAD4* (Aretz et al. 2007, Calva-Cerqueira et al. 2009, Latchford et al. 2012, van Hattem et al. 2008) *Based on a 2016-2024 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	Length (nt)	Probe number	Warnings
10q23.2	*BMPR1A*	Exon 1	0.3 kb	222	11840-L31344	#
10q23.2	*BMPR1A*	Exon 1	82.0 kb	137	07189-L15131	
10q23.2	*BMPR1A*	Exon 2	37.0 kb	218	21424-L29928	
10q23.2	*BMPR1A*	Exon 3	0.1 kb	275	05131-L07338	#
10q23.2	*BMPR1A*	Exon 3	14.1 kb	285	07191-L07339	
10q23.2	*BMPR1A*	Exon 4	2.1 kb	258	22025-L30948	#
10q23.2	*BMPR1A*	Exon 5	7.7 kb	355	19659-L26347	
10q23.2	*BMPR1A*	Exon 6	0.1 kb	383	11844-L19281	
10q23.2	*BMPR1A*	Exon 7	12.4 kb	310	11843-L12640	+
10q23.2	*BMPR1A*	Exon 8	4.9 kb	172	16647-L19180	+
10q23.2	*BMPR1A*	Exon 9	1.8 kb	328	07196-L06811	
10q23.2	*BMPR1A*	Exon 10	2.3 kb	160	07197-L06812	
10q23.2	*BMPR1A*	Exon 11	2.0 kb	211	16649-L19182	
10q23.2	*BMPR1A*	Exon 12	0.6 kb	337	07199-L06814	
10q23.2	*BMPR1A*	Exon 13	937.7 kb	454	05138-L07343	#
10q23.31	*PTEN*	Promoter	2.8 kb	184	11280-L15522	Ø
10q23.31	*PTEN*	Exon 1	29.8 kb	472	17394-L29893	
10q23.31	*PTEN*	Intron 2 (Exon 2)	31.3 kb	190	06729-L06339	Ø
10q23.31	*PTEN*	Exon 3	0.1 kb	481	16652-L19185	+
10q23.31	*PTEN*	Intron 3 (Exon 3)	5.4 kb	178	16648-L19181	Ø
10q23.31	*PTEN*	Exon 4	2.1 kb	229	03718-L02944	
10q23.31	*PTEN*	Exon 5	19.1 kb	376	03638-L25975	#
10q23.31	*PTEN*	Exon 6	5.7 kb	319	03639-L02946	
10q23.31	*PTEN*	Exon 7	3.3 kb	148	19343-L25668	#
10q23.31	*PTEN*	Exon 8	6.9 kb	400	21288-L02947	# +
10q23.31	*PTEN*	Exon 9		436	16651-L19278	
18q21.2	*SMAD4*	Upstream (Exon 1)	0.3 kb	124	S0151-L14963	Ø
18q21.2	*SMAD4*	Exon 1	0.4 kb	166	07796-L08332	
18q21.2	*SMAD4*	Exon 1	0.2 kb	463	07798-L07553	
18q21.2	*SMAD4*	Intron 1 (Exon 1)	16.2 kb	418	07797-L19282	Ø
18q21.2	*SMAD4*	Exon 2	1.6 kb	142	02127-L01638	∫
18q21.2	*SMAD4*	Exon 3	0.6 kb	266	05142-L07337	∫
18q21.2	*SMAD4*	Exon 4	5.6 kb	346	05143-L04533	+
18q21.2	*SMAD4*	Exon 5	3.3 kb	409	16522-L31346	∫
18q21.2	*SMAD4*	Exon 6	0.2 kb	445	05145-L07344	∫
18q21.2	*SMAD4*	Exon 7	1.5 kb	238	21420-L30664	
18q21.2	*SMAD4*	Exon 8	5.6 kb	196	05147-L07333	∫
18q21.2	*SMAD4*	Exon 9	1.6 kb	244	11841-L29917	∫
18q21.2	*SMAD4*	Exon 10	9.6 kb	301	11842-L12639	∫
18q21.2	*SMAD4*	Exon 11	1.7 kb	362	07799-L26846	∫
18q21.2	*SMAD4*	Exon 12		427	07800-L07555	∫
1q	Reference			252	20527-L28117	
5q	Reference			130	00797-L00463	
5q	Reference			118	19041-L24884	
7q	Reference			203	04732-L04149	
14q	Reference			499	14882-L21050	
15q	Reference			154	03931-L03386	
17q	Reference			370	08326-L22797	
18p	Reference			490	14909-L17529	
20p	Reference			391	11958-L19280	
21q	Reference			292	03796-L03237	

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 *BMPR1A, PTEN,* and *SMAD4* exon numbers are derived from the MANE Project and are based on MANE Select transcripts. 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 specificity of these probes rely 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 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.

Ø These probes target a sequence outside of the known coding region. Copy number alterations of only one of these probes are of unknown clinical significance.

- A recurrent duplication of *SMAD4* exons has been reported (Millson et al. 2015; Mancini et al. 2015). This is due to a processed *SMAD4* pseudogene which is present in ~0.25% of the population that affects exons 2-12 with the exception of exon 4 and 7 probe ratios. Exon 1 and intron 1 probes are not duplicated either in this case. This pseudogene probably has no clinical significance. Since the publication of this manuscript, the probe for exon 7 has been altered, and is therefore no longer duplicated as described in the publication.

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. Before testing patient samples, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.

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. Aretz S et al. (2007). High proportion of large genomic deletions and a genotype phenotype update in 80 unrelated families with juvenile polyposis syndrome. J Med Genet. 44:702-709.
2. Calva-Cerqueira D et al. (2009). The rate of germline mutations and large deletions of SMAD4 and BMPR1A in juvenile polyposis. Clin Genet. 75:79-85.
3. Dahdaleh FS et al. (2012). Juvenile polyposis and other intestinal polyposis syndromes with microdeletions of chromosome 10q22–23. Clin Genet. 81:110-116.
4. Latchford AR et al. (2012). Juvenile polyposis syndrome: a study of genotype, phenotype, and long-term outcome. Dis Colon Rectum. 55:1038-1043.
5. van Hattem WA et al. (2008). Large genomic deletions of SMAD4, BMPR1A and PTEN in juvenile polyposis. Gut. 57:623-627.
6. Millson A et al. (2015). Processed pseudogene confounding deletion/duplication analysis assays for SMAD4. J Mol Diagnostics 17:1-7.
7. Mancini et al. (2015). Dosage analysis by next generation sequencing and microarray CGH indicates putative processed pseudogenes in SMAD4 and NBN. Presented at ACMG 2015 (also see https://www.myriadpro.com/for-your-practice/myriad-publications/).

Implemented changes in the product description

Version D1-05 – 24 March 2025 (03S)

- Product description updated to new template.
- Intended purpose updated by limiting the CNV type detected in the *BMPR1A* and *SMAD4* genes to deletions. It has further been specified that the confirmation of a clinical diagnosis can take place in the case of inconclusive clinical features.
- SNVs rs147586703 and rs146326040 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Warning for a ligation site >20nt away from the nearest exon added to probes 11843-L12640, 16647-L19180, 16652-L19185, 21288-L02947, and 05143-L04533.
- Warning for target outside the transcript region added for probes 11280-L15522, 06729-L06339, 16648-L19181, S0151-L14963, and 07797-L19282.
- Salt warning removed for probes 11840-L31344, 07189-L15131, S0151-L14963, 07796-L08332, 07798-L07553, and 07797-L19282.
- Performance Characteristics section updated based on analytical performance experiments.
- Probemix is now IVDR-certified.