Product Description
SALSA® MLPA® Probemix P118-C3 WT1

To be used with the MLPA General Protocol.

Version C3
For complete product history see page 7.

Catalogue numbers:
- **P118-025R:** SALSA MLPA Probemix P118 WT1, 25 reactions.
- **P118-050R:** SALSA MLPA Probemix P118 WT1, 50 reactions.
- **P118-100R:** SALSA MLPA Probemix P118 WT1, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information
The SALSA MLPA Probemix P118 WT1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *WT1* gene region and the *AMER1* gene, which are associated with Wilms tumours.

The majority of Wilms tumour, or nephroblastoma, is caused by defects in the *WT1* gene on chromosome 11p13. The *WT1* gene product is required for normal formation of the genitourinary system and mesothelial tissues. Mutations in *WT1* gene have also been observed in patients with Denys-Drash syndrome, Frasier syndrome, Meacham syndrome, and type 4 nephrotic syndrome. Mutations in the *WT1* gene are observed in only 5% of the sporadic Wilms tumours. However, more than 90% of patients with Denys-Drash syndrome, which includes Wilms tumour, carry constitutional intragenic *WT1* mutations. Large chromosomal deletions including the *WT1* gene cause WAGR syndrome. We recommend the P219 PAX6 probemix to study the extent of chromosomal deletions in WAGR syndrome patients. Deletions at chromosome Xq11 have been implicated in sporadic Wilms tumours. The only gene in the interval is *AMER1*, which is also called WTX for 'Wilms tumour gene on the X chromosome'.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1294/.

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *WT1* and *AMER1* exon numbering used in this P118-C3 WT1 product description is the exon numbering from the MANE project (release version 1.0.1) based on MANE Select transcript as indicated in Table 2. The exon numbering of the MANE transcript that was used for determining a probe's ligation site does not always

correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P118-C3 WT1 contains 34 MLPA probes with amplification products between 130 and 409 nucleotides (nt). This includes 17 probes for the *WT1* gene region, one probe for each of the 10 exons and two probes for exon 1, two probes upstream of the gene, and four probes downstream of the gene. Furthermore, five probes for the *AMER1* gene region, one for exon 1, two for exon 2, one probe upstream, and one probe downstream of this gene, are also included. In addition, 12 reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals of the same sex is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. 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. Reference samples should be derived from different unrelated individuals who are from families without a history of Wilms tumours. It is recommended to use samples of the same sex to facilitate interpretation. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA05518, NA06803, and NA09709 from the Coriell Institute have been tested with this P118-C3 probemix at MRC Holland and can be used as positive

control samples to detect heterozygous deletion of the entire *WT1* gene. The flanking probes that are present in this probemix are also affected. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences in males and females, and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	Duplication	1.75 < FR < 2.15
Ambiguous copy number		All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.

- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *WT1* and *AMER1* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P118 WT1.
- 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.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) 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.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***WT1* mutation database**

https://databases.lovd.nl/shared/genes/WT1. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *WT1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P118-C3 WT1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*AMER1*	*WT1*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L13645	5q31		
136	Reference probe 13956-L15525	7q34		
142	**WT1 probe** 14805-L16513			**Exon 2 (3)**
148	Reference probe 15075-L18383	12q14		
153	**WT1 probe** 05357-L05466			**Exon 7 (8)**
160	**WT1 probe** 14806-L16514			**Exon 3 (4)**
166	**WT1 probe** 05358-L04737			**Exon 8 (9)**
172	Reference probe 07915-L07628	8q13		
178	**WT1 probe** 05354-L04733			**Exon 4 (5)**
185	**WT1 probe** 14807-L18521			**Exon 9 (10b)**
190 ¬	**WT1-area probe** 14795-L06713			Downstream of *WT1*
200 ¬	**HIPK3 probe** 00976-L07192			Upstream of *WT1*
211	Reference probe 04965-L04351	1p22		
220 ¬	**PAX6 probe** 03253-L02690			Downstream of *WT1*
231	Reference probe 10452-L11005	6p21		
239	**WT1 probe** 15025-L16774			**Exon 6 (7)**
247	**WT1 probe** 02755-L02204			**Exon 1**
265	**WT1 probe** 05355-L04734			**Exon 5 (6)**
274	**WT1 probe** 05360-L04739			**Exon 10 (11a)**
283	Reference probe 05695-L05137	12q24		
292 ¬	**WT1-area probe** 14808-L16516			Downstream of *WT1*
301	**WT1 probe** 14796-L02493			**Exon 1**
310	Reference probe 01230-L00059	4q24		
320 ¬	**LMO2 probe** 12984-L14141			Upstream of *WT1*
328 ¬	**PAX6 probe** 14809-L16517			Downstream of *WT1*
336	**AMER1 probe** 14801-L13868		**Exon 2**	
344	Reference probe 08604-L08612	10q26		
355	**AMER1 probe** 15331-L17133		**Exon 1**	
363 ¬	**ATP7A probe** 07477-L07134		Upstream of *AMER1*	
372	Reference probe 16852-L19646	18q21		
382	**AMER1 probe** 14802-L13876		**Exon 2**	
391	Reference probe 13603-L03531	17p13		
400 ¬	**RPGR probe** 13114-L14333		Downstream of *AMER1*	
409	Reference probe 09999-L10331	20q13		

[a] See section Exon numbering on page 1 for more information.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P118-C3 probes arranged according to chromosomal location

Table 2a. *AMER1*

Length (nt)	SALSA MLPA probe	*AMER1* exon[a]	Ligation site NM_152424.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
400 ¬	13114-L14333	*RPGR*		ATAACCTCCATT-TTGCCTTGTTCT	2.5 Mb
		stop codon	*3643-3645 (Exon 2)*		
382	14802-L13876	Exon 2	3244-3245	TGACCATGTCAA-TATCACTATCAG	2.7 kb
336	14801-L13868	Exon 2	517-518	TCAGCAAGAGCA-AGACCCACGATG	12.6 kb
		start codon	*238-240 (Exon 2)*		
355	15331-L17133	Exon 1	130-131	CTAGGAACCTGA-CCGGGCTGGGTA	1.4 Mb
363 ¬	07477-L07134	*ATP7A*		ATTGGGAGCTAT-TGATGTAGAACG	

Table 2b. *WT1 gene*

Length (nt)	SALSA MLPA probe	*WT1* exon[a]	Ligation site NM_024426.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
328 ¬	14809-L16517	*PAX6* Exon 8		GAAGGAGGGGGA-GAGAATACCAAC	8.1 kb
220 ¬	03253-L02690	*PAX6* Exon 5		GTGAATCAGCTC-GGTGGTGTCTTT	427.3 kb
292 ¬	14808-L16516	Downstream	158 kb after exon 10	AGGTCGCTCAAG-TTCTGCAGCTCC	88.2 kb
190 ¬	14795-L06713	Downstream	70 kb after exon 10	GCTTGTAGATCT-GTCCCTTGGCCT	70.2 kb
274	05360-L04739	Exon 10 (11a)	2276-2277	GTCAGCCAGGCT-GCTAACCTGGAA	3.6 kb
		stop codon	*1746-1748 (Exon 10)*		
185	14807-L18521	Exon 9 (10b)	21 nt before exon 9, reverse	CAGAGAAGGTCT-AGCCTCGGCCCT	0.6 kb
166	05358-L04737	Exon 8 (9)	1463-1464	CCATACCAGTGT-GACTTCAAGGAC	3.6 kb
153	05357-L05466	Exon 7 (8)	1354-1355	TGAGACCAGTGA-GAAACGCCCCTT	3.7 kb
239	15025-L16774	Exon 6 (7)	1220-1219, reverse	GTTGTGTGGTTA-TCGCTCTCGTAC	16.5 kb
265	05355-L04734	Exon 5 (6)	1169-1170	AGCTCCAGCTCA-GTGAAATGGACA	1.1 kb
178	05354-L04733	Exon 4 (5)	1098-1099	CATCCCAGCTTG-AATGCATGACCT	10.4 kb
160	14806-L16514	Exon 3 (4)	973-974	AGGTGAGCAGCA-GTACTCGGTGCC	0.4 kb
142	14805-L16513	Exon 2 (3)	30 nt after exon 2	GTGGAGTCCTTC-TCCCCTTCTTCC	6.4 kb
301	14796-L02493	Exon 1	685-686	CACTGTCCACTT-TTCCGGCCAGTT	0.9 kb
		start codon	*180-182 (Exon 1)*		
247	02755-L02204	Exon 1	217 nt before exon 1	CACCGGCCAGCT-GAGAGCGCGTGT	917.6 kb
200 ¬	00976-L07192	*HIPK3*		CAGCATCCAACT-TATAATATCTCC	516.2 kb
320 ¬	12984-L14141	*LMO2*		CCCGGTGATTCG-CTCTCTCTCTTT	

[a] See section Exon numbering on page 1 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P219 PAX6	Contains probes for *PAX6*, *WT1*, and WAGR region on 11p13.
ME030 BWS	Beckwith-Wiedemann syndrome. Constitutional 11p15 abnormalities have been identified in 3% of non-syndromic Wilms tumour cases with the use of this ME030 MS-MLPA probemix.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P118 WT1

- Busch M et al. (2013). Evaluation of chromosome 11p imbalances in aniridia and Wilms tumor patients. *Am J Med Genet* A. 161A:958-64.
- Haruta M et al. (2019). Combined genetic and chromosomal characterization of Wilms tumors identifies chromosome 12 gain as a potential new marker predicting a favorable outcome. *Neoplasia*, 21(1), 117-131.
- Hollink IH et al. (2009). Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia. *Blood*. 113:5951-60.
- Scott RH et al. (2012). Stratification of Wilms tumor by genetic and epigenetic analysis. *Oncotarget*, 3(3), 327.
- Segers H et al. (2012). Frequency of WT1 and 11p15 constitutional aberrations and phenotypic correlation in childhood Wilms tumour patients. *Eur J Cancer*. 48:3249-56.

P118 product history	
Version	*Modification*
C3	Four reference probes have been replaced.
C2	One reference probe has been replaced, and one added. In addition, the control fragments have been adjusted (QDX2).
C1	Three probes for the *FAM123B (AMER1)* gene have been included. The number of probes in the 11p13 region, but outside the *WT1* gene, has been strongly reduced.
B1	The *WT1* exon 3 probe has been replaced, one reference probe has been removed and four extra control fragments at 88, 96, 100, and 105 nt have been included.
A	First release.

Implemented changes in the product description

Version C3-02 – 28 October 2024 (04P)
- Product description rewritten and adapted to a new template.
- Exon numbering of the *WT1* gene has been adjusted to the exon numbering of the MANE Select transcript in Table 1 and 2.
- Ligation sites of the probes targeting the *WT1* and *AMER1* genes updated according to new version of the NM_ reference sequence.
- Reference added to the list of publications using SALSA MLPA Probemix P118 WT1.

More information: www.mrcholland.com; www.mrcholland.eu

	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200