Product Description SALSA® MLPA® Probemix P118-C3 WT1

To be used with the MLPA General Protocol.

Version C3. As compared to version C2, four reference probes have been replaced. For complete product history see page 6.

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, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

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

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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/

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 and surrounding region. Furthermore, it also contains three probes for the *AMER1* gene. In addition, 12 reference probes are included and detect 12 different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.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.mlpa.com.

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

No DNA controls result in only five major peaks shorter than 105 nt: four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 105 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

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

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: 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 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.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID numbers NA05518, NA06803 and NA09709 from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control samples to detect a deletion of the complete *WT1* gene. NA06803 and NA09709 are derived from male patients, therefore the probes targeting the X-chromosome will also show a ratio of ~0.5 in these samples. 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.mlpa.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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status for *WT1* (male and female) and *AMER1* (female)	Copy Number status for *AMER1* (male)	Dosage quotient
Normal	Normal	0.80 < DQ < 1.20
Homozygous deletion	Deletion	DQ = 0
Heterozygous deletion		0.40 < DQ < 0.65
Heterozygous duplication		1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	Duplication	1.75 < DQ < 2.15
Ambiguous copy number	Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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 (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 are unlikely to have any relation to the condition tested for.

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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=WT1
We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD) of the *WT1* gene. 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 SNPs and unusual results (e.g., a duplication of *WT1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P118-C3 WT1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		
		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 3**
148 *	Reference probe 15075-L18383	12q14		
153	**WT1 probe** 05357-L05466			**Exon 8**
160	**WT1 probe** 14806-L16514			**Exon 4**
166	**WT1 probe** 05358-L04737			**Exon 9**
172	Reference probe 07915-L07628	8q13		
178	**WT1 probe** 05354-L04733			**Exon 5**
185	**WT1 probe** 14807-L18521			**Exon 10**
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 7**
247	**WT1 probe** 02755-L02204			**Exon 1**
265	**WT1 probe** 05355-L04734			**Exon 6**
274	**WT1 probe** 05360-L04739			**Exon 11**
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		

* New in version C3 (from lot C3-0418 onwards).
¬ 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.

Note: The exon numbering used for *WT1* in this P118-C3 WT1 product description is the exon numbering from the LRG_525 sequence, which is identical to NG_009272.1. RefSeq transcript NM_024426.4, which represents transcript variant D, is used to indicate the ligation sites in Table 2b. The exon numbering and ligation sites used for *AMER1* are derived from the RefSeq transcript NM_152424.3. The exon numbering and NM sequences used are from 04/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

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

Table 2a. *AMER1*

Length (nt)	SALSA MLPA probe	*AMER1* exon	Ligation site NM_152424.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
400 ¬	13114-L14333	*RPGR*		ATAACCTCCATT-TTGCCTTGTTCT	2.5 Mb
		stop codon	*3679-3681 (Exon 2)*		
382	14802-L13876	Exon 2	3280-3281	TGACCATGTCAA-TATCACTATCAG	2.7 kb
336	14801-L13868	Exon 2	553-554	TCAGCAAGAGCA-AGACCCACGATG	12.6 kb
		start codon	*274-276 (Exon 2)*		
355	15331-L17133	Exon 1	166-167	CTAGGAACCTGA-CCGGGCTGGGTA	1.4 Mb
363 ¬	07477-L07134	*ATP7A*		ATTGGGAGCTAT-TGATGTAGAACG	

Table 2b. *WT1*

Length (nt)	SALSA MLPA probe	*WT1* exon	Ligation site NM_024426.4	Partial sequence (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 *WT1*	158 kb after exon 11	AGGTCGCTCAAG-TTCTGCAGCTCC	88.2 kb
190 ¬	14795-L06713	Downstream *WT1*	70 kb after exon 11	GCTTGTAGATCT-GTCCCTTGGCCT	70.2 kb
274	05360-L04739	Exon 11a	2272-2273	GTCAGCCAGGCT-GCTAACCTGGAA	3.6 kb
		stop codon	*1742-1744 (Exon 11a)*		
185	14807-L18521	Exon 10b	20 nt before exon 10b reverse	CAGAGAAGGTCT-AGCCTCGGCCCT	0.6 kb
166	05358-L04737	Exon 9	1459-1460	CCATACCAGTGT-GACTTCAAGGAC	3.6 kb
153	05357-L05466	Exon 8	1350-1351	TGAGACCAGTGA-GAAACGCCCCTT	3.7 kb
239	15025-L16774	Exon 7	1216-1215 reverse	GTTGTGTGGTTA-TCGCTCTCGTAC	16.5 kb
265	05355-L04734	Exon 6	1165-1166	AGCTCCAGCTCA-GTGAAATGGACA	1.1 kb
178	05354-L04733	Exon 5	1094-1095	CATCCCAGCTTG-AATGCATGACCT	10.4 kb
160	14806-L16514	Exon 4	969-970	AGGTGAGCAGCA-GTACTCGGTGCC	0.4 kb
142	14805-L16513	Exon 3	30 nt after exon 3	GTGGAGTCCTTC-TCCCCTTCTTCC	6.4 kb
301	14796-L02493	Exon 1	681-682	CACTGTCCACTT-TTCCGGCCAGTT	0.9 kb
		start codon	*191-193 (Exon 1)*		
247	02755-L02204	Exon 1	221 nt before exon 1	CACCGGCCAGCT-GAGAGCGCGTGT	917.6 kb
200 ¬	00976-L07192	*HIPK3*		CAGCATCCAACT-TATAATATCTCC	516.2 kb
320 ¬	12984-L14141	*LMO2*		CCCGGTGATTCG-CTCTCTCTCTTT	

¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication.

Note: The exon numbering used for *WT1* in this P118-C3 WT1 product description is the exon numbering from the LRG_525 sequence, which is identical to NG_009272.1. RefSeq transcript NM_024426.4, which represents transcript variant D, is used to indicate the ligation sites in Table 2b. The exon numbering and ligation sites used for *AMER1* are derived from the RefSeq transcript NM_152424.3. The exon numbering and NM sequences used are from 04/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

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.
- Hollink IH et al. (2009). Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia. *Blood*. 113:5951-60.
- 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 C3-01 – 17 May 2018 (01P) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1). *Version 06 -20 January 2015 (54)* - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1, new picture included). - Various small textual changes. *Version 05 (53)* - Gene name changes on page 1 and in Table 1 and Table 2.

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