Product Description SALSA® MLPA® Probemix P258-C2 SMARCB1

To be used with the MLPA General Protocol.

Version C2. For complete product history see page 8.

Catalogue numbers:
- **P258-025R:** SALSA MLPA Probemix P258 SMARCB1, 25 reactions.
- **P258-050R:** SALSA MLPA Probemix P258 SMARCB1, 50 reactions.
- **P258-100R:** SALSA MLPA Probemix P258 SMARCB1, 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.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 P258 SMARCB1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SMARCB1* gene, inactivation of which has been associated with malignant rhabdoid tumours (MRTs).

Rhabdoid tumours are a highly malignant group of neoplasms that usually occur in children under two years of age. MRTs of the kidney were first identified as a sarcomatous variant of Wilms tumours (Beckwith and Palmer, 1978, *Cancer.* 41:1937-48). Later, extrarenal rhabdoid tumours were reported in numerous locations, including the central nervous system (Parham et al., 1994, *Am J Surg Pathol.* 18:474-8).

The protein encoded by the *SMARCB1* gene (also known as INI1 or SNF5) is part of a complex that relieves repressive chromatin structures, allowing the transcriptional machinery to access its targets more effectively. The encoded nuclear protein may also bind to and enhance the DNA joining activity of HIV-1 integrase. The *SMARCB1* gene has been characterised as a tumour suppressor and is frequently deleted in malignant rhabdoid tumours.

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 *SMARCB1* exon numbering used in this P258-C2 SMARCB1 product description is the exon numbering from the RefSeq transcript NM_003073.3, which is identical to the LRG_520 transcript t1. The exon numbering and NM_ sequence used have been retrieved on 07/2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P258-C2 SMARCB1 contains 42 MLPA probes with amplification products between 127 and 445 nucleotides (nt). This includes two probes for each exon of the *SMARCB1* gene (nine exons). This probemix furthermore contains 10 probes flanking SMARCB1 in 22q11 and 22q12 chromosomal regions. In addition, 14 reference probes are included detecting several different

autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 2 and 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 nt and 96 nt fragment 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.mlpa.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals 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 reference probes over the experiment.

Required specimens: Extracted DNA, which includes DNA derived from paraffin-embedded tissues, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

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 healthy individuals without a history of rhabdoid tumours and other SMARCB1-related syndromes and cancers. 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 Institute (https://catalog.coriell.org) and Leibniz Institute 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 number(s) NA02325 and NA07106 from the Coriell Institute have been tested with this P258-C2 probemix at MRC-Holland and can be used as positive control samples to detect heterozygous duplications in the *SMARCB1* gene and in the surrounding genomic regions targeted by flanking probes. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA	Altered target genes in P258-C2	Expected CNA
NA02325	Coriell Institute	22q11.21-q12.2	*TBX1, DGCR8, SNAP29, LZTR1, PPIL2, GNAZ,* ***SMARCB1****, SNRPD3, SEZ6L, NIPSNAP1*	Heterozygous duplication
NA07106	Coriell Institute	22q11.21-q12.2	*TBX1, DGCR8, SNAP29, LZTR1, PPIL2, GNAZ,* ***SMARCB1****, SNRPD3, SEZ6L, NIPSNAP1*	Heterozygous duplication

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 each individual reference probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

Please note that these above mentioned dosage quotients are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases.
- 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 (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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.
- Confirmation of deletions, duplications and amplifications can be done by e.g. Southern blotting, long range PCR, qPCR, FISH.

Limitations of the procedure:

- In most populations, the most genetic alterations in the *SMARCB1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P258 SMARCB1.
- 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.

- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in tumours with more chaotic karyotypes. **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.

COSMIC mutation database: http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the Catalogue Of Somatic Mutations In Cancer (COSMIC). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNPs and unusual results (e.g., a duplication of *SMARCB1* exons 2 and 4 but not exon 3) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P258-C2 SMARCB1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		
		reference	22q11-q12 flanking probes	*SMARCB1*
64-105	Control fragments – see table in probemix content section for more information			
127 ¥	Reference probe 18709-L26552	5q31		
137	Reference probe 03797-L04594	21q22		
144 ¬	GNAZ probe 08477-L08488		22q11.22	
150 « ¬	DGCR8 probe 08475-L20825		22q11.21	
155	Reference probe 08375-L20826	15q24		
160	**SMARCB1 probe** 08283-L20827			**Exon 3**
166 ¬	**SNRPD3 probe** 08481-L20828		22q11.23	
172	**SMARCB1 probe** 08295-L08109			**Exon 9**
177 « ¬	TBX1 probe 05408-L19742		22q11.21	
184	**SMARCB1 probe** 08292-L20678			**Exon 7**
190	**SMARCB1 probe** 08287-L08101			**Exon 5**
196	**SMARCB1 probe** 16893-L19747			**Exon 2**
202	Reference probe 05706-L20677	3q21		
208	**SMARCB1 probe** 08288-L08102			**Exon 5**
214	**SMARCB1 probe** 08282-L08096			**Exon 2**
220	Reference probe 08879-L08935	2p23		
230 ¥	**SMARCB1 probe** 18230-L23153			**Exon 6**
238 ¥	Reference probe 14498- L23152	20p12		
245	**SMARCB1 probe** 08296-L19752			**Exon 9**
251 « ¬	TBX1 probe 10810-L19753		22q11.21	
256	**SMARCB1 probe** 08284-L08098			**Exon 3**
265	**SMARCB1 probe** 08286-L08100			**Exon 4**
275 ¬	SEZ6L probe 05929-L05810		22q12.1	
283	**SMARCB1 probe** 08289-L08103			**Exon 6**
292	**SMARCB1 probe** 08293-L08107			**Exon 8**
301	Reference probe 12783-L13918	2q13		
310	**SMARCB1 probe** 08280-L20836			**Exon 1**
319	**SMARCB1 probe** 08294-L20837			**Exon 8**
328	Reference probe 04007-L03430	2q33		
336	**SMARCB1 probe** 08279-L08093			**upstream**
346	Reference probe 06560-L06118	1q32		
355	**SMARCB1 probe** 08285-L19741			**Exon 4**
364	**SMARCB1 probe** 08291-L08105			**Exon 7**
373 ¬	SNAP29 probe 16748-L19368		22q11.21	
382 *	Reference probe 01522-L00952	10p14		
391 « ¬	NIPSNAP1 probe 02580-L02042		22q12.2	
400	Reference probe 10670-L11252	6p12		
409 ¬	PPIL2 probe 05467- L20675		22q11.21	
418 ¬	LZTR1 probe 01521-L00951		22q11.21	
427	Reference probe 06942-L06522	11q12		
436	Reference probe 09077-L09246	19p13		
445	Reference probe 08793-L08817	10q21		

* New in version C2.
¥ Changed in version C2. Minor alteration, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ 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.

Table 2. P258 probes arranged according to chromosomal location

Table 2a. Target probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene/ Exon	Location/ Ligation site	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
Flanking probes centromeric to *SMARCB1* gene					
177 « ¬	05408-L19742	TBX1 exon 2	22q11.21	CCGGGTGAAGCT-TCGCTGGCTGCC	6.2 kb
251 « ¬	10810-L19753	TBX1 exon 7	22q11.21	TCCCTTCGCGAA-AGGCTTCCGGGA	320.3 kb
150 « ¬	08475-L20825	DGCR8	22q11.21	GGTAATGGACGT-TGGCTCTGGTGG	1111.8 kb
373 ¬	16748-L19368	SNAP29	22q11.21	GTATCCACTTAC-CTGTATCATCCA	113.8 kb
418 ¬	01521-L00951	LZTR1	22q11.21	ATGATGAAGGAG-TTCGAGCGCCTC	700.5 kb
409 ¬	05467-L20675	PPIL2	22q11.21	GAAGAGCCCTCA-ACCAGTGCCACT	1388.2 kb
144 ¬	08477-L08488	GNAZ	22q11.22	TGACCGCCACCT-GCGCTCAGAGAG	690.9 kb
SMARCB1 gene, at 22q11.23. Indicated ligation sites are in NM_003073.5[b]					
		start codon	*205-207 (ex 1)*		
336	08279-L08093	upstream	224 nt before exon 1	ACCACCCAGGCT-TCCAGATACTAG	0.5 kb
310	08280-L20836	exon 1	226-227	TGGCGCTGAGCA-AGACCTTCGGGC	4.7 kb
196	16893-L19747	exon 2	25 nt after exon 2	AGGGTTTGTAAA-CCTGTTTCAAAA	0.2 kb
214	08282-L08096	exon 2	277 nt after exon 2, reverse	TCCTAAAACGTT-TTGAGACCAACA	1.4 kb
160	08283-L20827	exon 3	455-456	ATACACGACTCT-AGCCACCAGTGT	0.1 kb
256	08284-L08098	exon 3	531-530, reverse	CTGATGGACACA-GCCTTGTACTTC	7.4 kb
355	08285-L19741	exon 4	632-633	CCACCACTTAGA-TGCCGTGCCATG	0.1 kb
265	08286-L08100	exon 4	44 nt after exon 4, reverse	GCTGGAGAACTA-AGGCGGAATCAG	2.2 kb
190	08287-L08101	exon 5	730-731	ACCCAGCTGTGA-TCCATGAGAACG	0.1 kb
208	08288-L08102	exon 5	814-815	TGCGAGACGCCT-TCACCTGGAACA	13.4 kb
283	08289-L08103	exon 6	856-857	CGCCTGAGATGT-TTTCAGAAATCC	0.1 kb
230	18230-L23153	exon 6	997-998	GCGTCATCATCA-AGGTAGGTGACT	8.3 kb
364	08291-L08105	exon 7	1018-1019	TCCATGTGGGAA-ACATTTCCCTGG	0.1 kb
184	08292-L20678	exon 7	1147-1148	TCGCATACAGCA-TCCGGGGACAGC	8.2 kb
292	08293-L08107	exon 8	1196-1195, reverse	TGGGCAGAGGGT-TCTCGCTACGAG	0.1 kb
319	08294-L20837	exon 8	1291-1292	CTGAGATGGAGA-AGAAGATCCGCG	0.6 kb
172	08295-L08109	exon 9	1453-1452, reverse	TGGCGCTGGGCT-GTCCCCTCGCCT	0.3 kb
245	08296-L19752	exon 9	93 nt after exon 9	GGTATGTGAACA-AGGTTGGCACAC	776.9 kb
		stop codon	*1360-1362 (ex 9)*		
Flanking probes telomeric to *SMARCB1* gene					
166 ¬	08481-L20828	SNRPD3	22q11.23	CCGGTGAGGTAT-ATCGGGGGAAGC	1734.8 kb
275 ¬	05929-L05810	SEZ6L	22q12.1	ACAGTCGGCGGA-AGTGCTGGGCGA	3263.2 kb
391 « ¬	02580-L02042	NIPSNAP1	22q12.2	AGGCTGACAAGT-TCTGAGGATTAC	

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

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ 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.

Table 2b. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Location (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
346	06560-L06118	TNNT2	1q32	TTGAGAGAAACG-AGCTCCTCCTCC	01-199.601
220	08879-L08935	POMC	2p23	TCAGCCTCTTAA-AGCTGCCTGTAG	02-025.237
301	12783-L13918	EDAR	2q13	CTCCACACACGT-TGGCATACACAT	02-108.889
328	04007-L03430	BMPR2	2q33	AGATGAGACAAT-AATCATTGCTTT	02-203.087
202	05706-L20677	CASR	3q21	GATACAGGATAT-TTGACACTTGCA	03-123.459
127	18709-L26552	IL4	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132.038
400	10670-L11252	PKHD1	6p12	TTCAGTTGGTCA-GAGGAACCAAGG	06-052.033
382 *	01522-L00952	CELF2	10p14	TCCCCCGGTCAT-GGTCGGAAAAGG	10-011.248
445	08793-L08817	PCDH15	10q21	AGGAGGACGCAT-TCTGGAGATCCG	10-055.261
427	06942-L06522	BEST1	11q12	GACGAGGAGGAT-GCTCACGCTGGC	11-061.486
155	08375-L20826	CSK	15q24	TTTCGGAATCCT-TCTCTGGGAAAT	15-072.881
436	09077-L09246	CACNA1A	19p13	GATCGCCATGCT-CTTCTTCATCTA	19-013.207
238	14498-L23152	JAG1	20p12	AGGAGGCGTCAT-TCTGACACTGGC	20-010.578
137	03797-L04594	KCNJ6	21q22	CTCGAAGCTCCT-ACATCACCAGTG	21-037.920

Complete probe sequences are available at www.mlpa.com.

* New in version C2.

Related SALSA MLPA probemixes

– **P250 DiGeorge**: Contains more probes in the 22q11 region.
– **P324 22q11**: Contains more probes in the 22q11 region for research only.
– **P294 Tumour Loss**: Contains two probes for *SMARCB1*, among other genes.

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P258 Product history	
Version	*Modification*
C2	One reference probe replaced.
C1	One target probe, several reference probes and one flanking probe replaced. Two *TBX1* probes added. 88 and 96nt control fragments replaced (QDX2).
B1	Three reference probes and two flanking probes replaced.
A1	First lot of this product.

Implemented changes in the product description

Version C2-01 — 30 August 2019 (02P)
- Product description rewritten and adapted to a new template.
- Various minor textual and layout changes.
- Ligation sites of the probes targeting the *SMARCB1* gene updated according to LRG_520.
- Positive cell line sample information included on page 2.
- Table 2b (reference probes arranged according to chromosomal location) added.
- New references added on pages 9-10.

Version 08 – 28 January 2016 (T08)
- Product description adapted to a new lot (lot number added, small changes in Tables 1 and 2, new picture included).
- Various minor textual changes.
- References added on page 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the

amplification products.
- Exon numbering, NM_sequence and ligation sites updated for the *SMARCB1* gene in Table 1 and 2.

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- New references included on page 2.

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