

Product Description

SALSA® MLPA® Probemix P478-A1 SMARCE1

To be used with the MLPA General Protocol.

Version A1

New product. For complete product history see page 7.

Catalogue numbers:

- **P478-025R:** SALSA MLPA Probemix P478 SMARCE1, 25 reactions.
- **P478-050R:** SALSA MLPA Probemix P478 SMARCE1, 50 reactions.
- **P478-100R:** SALSA MLPA Probemix P478 SMARCE1, 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 P478 SMARCE1 is a **research use only (RUO)** assay for the detection of deletions or duplications of the *SMARCE1* gene, which are associated with familial meningioma and Coffin-Siris syndrome.

Meningiomas are slow-growing tumours that stem from the meninges. They are the most commonly occurring intracranial neoplasms, accounting for up to 30% of all brain tumours (Starr and Cha, 2017). The *SMARCE1* (Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1) gene encodes a component of the SWI/SNF complex, which modulates eukaryotic gene expression and DNA repair via nucleosome remodelling. Heterozygous *SMARCE1* loss-of-function mutations have been linked to familial meningioma (MIM number 607174). Partial deletions of *SMARCE1* and loss of its expression have been described in spinal meningioma tissue suggesting a tumour suppressor function of this gene (Smith et al. 2013; Smith et al. 2014). Germline mutations in members of SWI/SNF complex, including *SMARCE1*, have been described also in Coffin-Siris syndrome (MIM number 616938) (Tsurusaki et al. 2014).

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>

Exon numbering

The *SMARCE1* exon numbering used in this P478-A1 SMARCE1 product description is the exon numbering from the NG_032163.1 sequence. 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 P478-A1 SMARCE1 contains 37 MLPA probes with amplification products between 130 and 391 nucleotides (nt). This includes 15 probes for the *SMARCE1* gene at 17q21.2, as well as

seven flanking probes to help determine the extent of a copy number alteration detected in a sample. In addition, 15 reference probes are included that target relatively copy number stable regions in meningioma, as well as in somatic cancer samples in general. Probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and 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). 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 probes over the experiment.

Required specimens

Extracted DNA, derived from germline blood samples, or from tumour tissue and corresponding healthy tissue, 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. Information on the use of formalin-fixed paraffin-embedded 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 for germline analysis should be derived from different unrelated individuals who are from families without a history of Coffin-Siris syndrome, and for tumour analysis from different healthy individuals without a history of meningioma. 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. Adenocarcinoma cell line MFE-280, with sample ID number ACC-410 from the DSMZ has been tested with this P478-A1 probemix at MRC Holland and can be used as a positive control sample to detect a gain of *SMARCE1* and its flanking region. Note: a heterozygous duplication for the reference probes targeting chromosome arm 2p was also detected in this sample, as well as a

heterozygous deletion for the reference probe targeting 16q. 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 . When this criterion is fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	$0.80 < FR < 1.20$
Homozygous deletion	FR = 0
Heterozygous deletion	$0.40 < FR < 0.65$
Heterozygous duplication	$1.30 < FR < 1.65$
Heterozygous triplication/homozygous 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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios for somatic tissue samples 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 or subclonal 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. 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.

P478 specific note

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Limitations of the procedure

- In most populations, the major cause of Coffin-Siris syndrome-related genetic defects in the *SMARCE1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P478 *SMARCE1*.
- 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.
- 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 derived from tumour tissue, especially in solid 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.

LOVD, ClinVar and COSMIC mutation databases

[http:// SMARCE1.lovd.nl/genes/](http://SMARCE1.lovd.nl/genes/); <https://www.ncbi.nlm.nih.gov/clinvar/>; <http://cancer.sanger.ac.uk/cosmic>.

We strongly encourage users to deposit germline positive results in the LOVD and ClinVar databases or for somatic mutations in the COSMIC database. 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 SNVs and unusual results (e.g., a duplication of *SMARCE1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P478-A1 SMARCE1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a		Location (hg18) in kb
		Reference	Target region	
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 19616-L26704	4p13		04-042.278
136	Reference probe 17516-L21415	2q32		02-189.569
142	SMARCE1 probe 21681-L30338		Exon 1	17-036.058
150	Reference probe 10301-L30389	11q14		11-077.639
154	SMARCE1 probe 21682-L30339		Upstream	17-036.058
160	Reference probe 16253-L21825	19p13		19-012.779
166	SMARCE1 probe 21683-L30340		Exon 4	17-036.052
172	Reference probe 08726-L25038	9q21		09-078.038
178	SMARCE1 probe 21684-L30341		Exon 8	17-036.042
184 ∅	SMARCE1 probe 21685-L30342		Intron 4	17-036.051
190	Reference probe 18316-L23369	15q24		15-072.494
196	SMARCE1 probe 21686-L30343		Exon 5	17-036.047
211	Reference probe 15521-L17376	16q13		16-055.505
220 ~	Flanking probe 06971-L06551		17p13.2	17-006.272
226	SMARCE1 probe 21688-L30345		Exon 2	17-036.056
232	Reference probe 14739-L30804	4q22		04-089.187
238 ~	Flanking probe 21689-L30520		17q21.2	17-036.111
244	SMARCE1 probe 21690-L30805		Exon 9	17-036.041
250 ~	Flanking probe 08590-L30521		17p11.2	17-017.072
256	Reference probe 05409-L04215	5p13		05-037.012
263	SMARCE1 probe 21691-L30348		Exon 7	17-036.046
268	SMARCE1 probe 21692-L30349		Exon 11	17-036.035
274 ~	Flanking probe 21693-L30781		17q21.2	17-035.966
280	SMARCE1 probe 21687-L30780		Exon 10	17-036.041
286	SMARCE1 probe 21694-L30806		Exon 6	17-036.046
295	Reference probe 20124-L27681	3p12		03-081.667
301	Reference probe 10783-L28289	2p25		02-003.571
310 ~	Flanking probe 08568-L08569		17q23.2	17-057.294
319	SMARCE1 probe 21695-L30352		Exon 11	17-036.039
328	Reference probe 07527-L21498	11p13		11-031.773
337	Reference probe 09227-L26255	5q23		05-126.200
346 ~	Flanking probe 21696-L30353		17q21.2	17-035.895
355 ~	Flanking probe 21697-L30354		17q21.2	17-036.072
364	SMARCE1 probe 21698-L30355		Exon 3	17-036.055
373 ∅	SMARCE1 probe 21699-L30356		Intron 10	17-036.040
382	Reference probe 02459-L01903	15q21		15-046.572
391	Reference probe 10468-L11021	2p11		02-085.636

^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.

∅ Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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. SMARCE1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/exon ^a	Location (hg18)/ Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
220 ~	06971-L06551	<i>AIP1</i>	17p13.2	CGAGGACCTGGA-CGAGCTGCAGAA	10.8 Mb
250 ~	08590-L30521	<i>FLCN</i>	17p11.2	TGGCATTGAGAT-GAACAGTCGGAT	18.8 Mb
346 ~	21696-L30353	<i>TNS4</i>	17q21.2	AGAGCAAGGTAA-AAGGAAGCACGA	70.9 kb
274 ~	21693-L30781	<i>CCR7</i>	17q21.2	AAGGTATGCCTG-TGTCAGATGAG	69.3 kb
SMARCE1 , located on 17q21.2. Ligation sites are according to NM_003079.5.					
268	21692-L30349	Exon 11	5050-5051	TTTCTAAGTGTG-AAATGATGGAAC	3.9 kb
		<i>stop codon</i>	1325-1327 (exon 11)		
319	21695-L30352	Exon 11	1124-1125	TTACAGAGGAGA-CACACCTTGAAG	1.3 kb
373 ∅	21699-L30356	Intron 10	474 nt after exon 10 reverse	AGGAAAATCAGT-ATACCTAGAAGT	0.7 kb
280	21687-L30780	Exon 10	917-918	AGTTGTGCGGTC-TGAAAAGTAGAAG	0.7 kb
244	21690-L30805	Exon 9	898-899	TTTAAACAATGAA-CTTAAAAGGGTA	0.8 kb
178	21684-L30341	Exon 8	634-635	TTCACTCCAGAT-TATGATGATGGC	3.6 kb
263	21691-L30348	Exon 7	620-621	TTCAGCCTGCTG-AAGATCCAGATG	0.6 kb
286	21694-L30806	Exon 6	329-330	TTCTTTTTAAGG-TCTGGGACCAAG	1.0 kb
196	21686-L30343	Exon 5	252-253	TTCGTAGGCATC-CTCTGGTATCAC	3.3 kb
184 ∅	21685-L30342	Intron 4	1544 nt after exon 4	GACTAGGAAGAG-AAAGTGTACTCC	1.5 kb
166	21683-L30340	Exon 4	9 nt after exon 4	ACGGTAGGAGAA-TCAACTATTACA	3.1 kb
364	21698-L30355	Exon 3	1 nt after exon 3	CTCCTGCAACAG-TAAGTTTTAATT	0.2 kb
		<i>start codon</i>	92-94 (exon 2)		
226	21688-L30345	Exon 2	81-82	ACTGAGAACTTA-ATCTTCCAAAAT	2.0 kb
142 «	21681-L30338	Exon 1	9 nt after exon 1	CGGGTGAGTGT-TCCAAGTGGGAC	0.5 kb
154	21682-L30339	Upstream	433 nt before exon 1	CCAGGAGCGGGC-TCAGCGGGAGTC	13.6 kb
355 ~	21697-L30354	<i>KRT222</i>	17q21.2	CTCTCCATGCTG-TGGTGAGAATGA	39.2 kb
238 ~	21689-L30520	<i>KRT24</i>	17q21.2	CGAGGAGGTAGG-AACATGTTCTAC	21.2 Mb
310 ~	08568-L08569	<i>BRIP1</i>	17q23.2	ACGGGTAAGCTT-TATAAGGAAAAGT	-

^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.

∅ Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
301	10783-L28289	<i>RNASEH1</i>	2p25	TCATTTCGGGATT-TATAGGCAATGA	02-003.571
391	10468-L11021	<i>GGCX</i>	2p11	TGCGGGTGTGAA-AAAGCTGGATGC	02-085.636
136	17516-L21415	<i>COL3A1</i>	2q32	TACTTCAGGGCA-TGCCCGGAAGTC	02-189.569
295	20124-L27681	<i>GBE1</i>	3p12	ACCGAGTTGGAA-CAGCATTGCCAG	03-081.667
130	19616-L26704	<i>ATP8A1</i>	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042.278
232	14739-L30804	<i>PKD2</i>	4q22	CGCATTCAAAA-CTACACTATTC	04-089.187
256	05409-L04215	<i>NIPBL</i>	5p13	CAAGTGCCTGTT-TTACAACAGAAC	05-037.012
337	09227-L26255	<i>LMNB1</i>	5q23	AGCACTCTGGAT-GATGGATTCCAC	05-126.200
172	08726-L25038	<i>PCSK5</i>	9q21	ACACCTGCCAGA-GATGCCAAGGAA	09-078.038
328	07527-L21498	<i>PAX6</i>	11p13	AGTTCCAACGGA-GAAGATTCAGAT	11-031.773
150	10301-L30389	<i>GAB2</i>	11q14	AGCCAGCACCTT-CTCCGAGAGCGC	11-077.639
382	02459-L01903	<i>FBN1</i>	15q21	CAGTGTCCCAGT-GGAATGACTTTG	15-046.572
190	18316-L23369	<i>SEMA7A</i>	15q24	TACCCACAGAGA-CCTTCCAGGTGG	15-072.494
211	15521-L17376	<i>SLC12A3</i>	16q13	GTGCTCACCTTT-TACTGCCAGTAA	16-055.505
160	16253-L21825	<i>RNASEH2A</i>	19p13	AGGACACGGACT-TTGTCGGCTGGG	19-012.779

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

Related SALSA MLPA probemixes

- **P044 NF2:** Probes for the *NF2* gene, which is implicated in somatic meningioma.
- **P225 PTEN:** Probes for the *PTEN* gene, which may also be implicated in familial meningioma.
- **P258 SMARCB1:** Probes for the *SMARCB1* gene, which is implicated in Coffin-Siris syndrome.
- **P433 ARID1A-ARID1B:** Probes for the *ARID1A* and *ARID1B* genes, which are implicated in Coffin-Siris syndrome.
- **P472 SUFU:** Probes for the *SUFU* gene, which is implicated in familial meningioma.

References

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P478 product history	
Version	Modification
A1	First release.

Implemented changes in the product description

Version A1-02 – 26 April 2022 (04P)

- Product description rewritten and adapted to a new template.
- Various minor textual or layout changes.
- Ligation sites of the probes targeting the name gene updated according to new version of the NM_003079 reference sequence.

Version A1-01 – 13 September 2018 (01P)

- Not applicable, new document.

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

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