Product Description SALSA® MLPA® Probemix P088-C2 Oligodendroglioma 1p-19q

To be used with the MLPA General Protocol.

Version C2. As compared to version C1, one reference probe is replaced, lengths of several probes are adjusted with no change in the sequences detected, one reference probes is replaced and sample DNA used for this probemix is changed from SD021 to SD054. For complete product history see page 9.

Catalogue numbers:
- **P088-025R:** SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q, 25 reactions.
- **P088-050R:** SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q, 50 reactions.
- **P088-100R:** SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q, 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 P088 Oligodendroglioma 1p-19q is a **research use only (RUO)** assay for the detection of deletions and duplications in the 1p and 19q chromosomal regions which are associated with oligodendroglioma. This probemix can also be used to detect the presence of p.R132C and p.R132H point mutations in *IDH1* gene and p.R172K and p.R172M point mutations in *IDH2* gene.

Oligodendrogliomas are central nervous system neoplasms derived from a subset of glial cells, known as oligodendrocytes. The diagnosis of oligodendroglioma is based on the presence of both an *IDH* gene family mutation in combination with co-deletion of 1p and 19q arms (Louis et al. 2016).

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 P088-C2 Oligodendroglioma 1p-19q contains 58 MLPA probes with amplification products between 122 and 504 nucleotides (nt). This includes 19 probes for the 1p arm plus three flanking probes for 1q arm, and 11 probes for the 19q arm plus two flanking probes for 19p arm. Furthermore, it also contains four probes specific for the p.R132H and p.R132C mutation of *IDH1* and for the p.R172K and p.R172M mutation of *IDH2* which will only generate a signal when the mutation is present. In addition, 14 reference probes are included and target relatively copy number stable regions in central nervous system tumours, especially in oligodendrogliomas. Complete probe sequences and the identity of the genes detected by the reference probes is available in Table 2b.

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 nt 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 121 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 121 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 (50-250 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola 2012.

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 derived DNA for MLPA can be found in Atanesyan et al. 2017.

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 healthy individuals. 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 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. For example, samples from the Coriell Institute have been tested at MRC-Holland with the P088-C2 probemix; a heterozygous deletion of 1p36.32 (*GNB1, TNFRSF14 and TP73*) and a heterozygous duplication of *CDKN2A* and *CDKN2B* can be detected in NA22976, a heterozygous deletion of 1p36.23 (*PARK7*) can be found in NA50276 and a heterozygous deletion of 1q31.3-1q32.1 (*CRB1* and *TNNT2*) is present in NA00214. The quality of cell lines can change, therefore samples should either be acquired from quality assured biorepositories with minimal cell passage or should be validated before use.

SALSA Binning DNA SD054: The SD054 Binning DNA provided with this probemix can be used as Binning DNA sample for binning of four mutation-specific probes (203 nt probe 19529-L16492 *IDH1* p.R132H=c.395G>A mutation, 227 nt probe 14787-L23353 *IDH1* p.R132C=c.394C>T mutation, 238 nt probe 20963-L29002 *IDH2* p.R172K=c.515G>A mutation and 244 nt probe 20963-L29001 *IDH2* p.R172M=c.515G>T mutation). SD054 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequence detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD054 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signals, as for this purpose true mutation positive patient samples or cell lines should be used. It is strongly advised to use DNA sample and reference DNA samples extracted with the same method and derived from the same source of tissue. For further details, please consult the SD054 Binning DNA product description provided. **This product is for research use only (RUO).**

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. When this criterion is fulfilled, the following cut-off values for the dosage quotient (DQ) of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

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 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. 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.

P088 specific note:

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

Limitations of the procedure:

- In most populations, the most genetic alterations in 1p/19q chromosomal regions are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q.
- 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 solid tumours with 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 to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P088-C2 Oligodendroglioma 1p-19q

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)					Location (hg18) in kb
		Reference	Chr. 1	Chr. 19	*IDH* mutation	*CDKN2A/B*	
64-105	Control fragments – see Table in probemix content section for more information						
122	Reference probe 02844-L02274	18q11					18-019.394
131	**NOTCH2 probe** 05745-L05183		1**p**12				01-120.331
137	Reference probe 09957-L20646	17p13					17-007.355
142	**CDKN2B probe** 11867-L23298					exon 1	09-021.999
148 ¬	**SMARCA4 probe** 02488-L22890			19p13.2			19-011.031
153	**CDKN2A probe** 16881-L23102					exon 3	09-021.961
157	**UPK1A probe** 18116-L23103			19**q**13.12			19-040.856
163	**PTAFR probe** 18115-L23104		1**p**35.3				01-028.350
168 «	**CCNE1 probe** 02881-L23105			19**q**12			19-035.005
173	Reference probe 15449-L23605	12q13					12-046.676
178	**GNB1 probe** 02890-L20648		1**p**36.33				01-001.747
184	**PDCD5 probe** 02882-L02349			19**q**13.11			19-037.764
190	**CDKN2A probe** 16880-L20211					exon 1	09-021.984
196 ¬	**TNNT2 probe** 06557-L20938		1q32.1				01-199.604
203 § ¥	**IDH1 probe** 19529-L16492				**p.R132H**		02-208.821
208	Reference probe 16261-L18553	20q11					20-034.979
214 ¬	**LDLR probe** 02314-L20213			19p13.2			19-011.077
220	**PPP1R15A probe** 02887-L02354			19**q**13.33			19-054.070
227 ¥ §	**IDH1 probe** 14787-L23353				**p.R132C**		02-208.821
232	**CDKN2B probe** 10337-L23606					exon 2	09-021.996
238 § ¥	**IDH2 probe** 20963-L29002				**p.R172K**		15-088.433
244 § ¥	**IDH2 probe** 20963-L29001				**p.R172M**		15-088.433
253 ¥	**CDKN2A probe** 16060-L29163					exon 2	09-021.965
259	**GTF2B probe** 02871-L19715		1**p**22.2				01-089.126
265 Ж «	**UPK1A probe** 18117-SP0616-L23106			19**q**13.12			19-040.861
270	Reference probe 16659-L19210	2p16					02-051.107
277 *	Reference probe 17450-L29159	16p13					16-009.761
283 «	**CDKN2C probe** 18565-L24220		1**p**33				01-051.208
288	**FAF1 probe** 02877-L24219		1**p**33				01-051.026
293 «	**WNT4 probe** 06055-L24329		1**p**36.12				01-022.329
300	**BAX probe** 00348-L00174			19**q**13.33			19-054.151
306 ¬	**LMNA probe** 16877-L19710		1q22				01-154.372
313 ¥ «	**CHMP2A probe** 18119-L29136			19**q**13.43			19-063.757
319 ¥	Reference probe 04833-L22803	5p13					05-037.032
326 «	**CHMP2A probe** 18118-L23300			19**q**13.43			19-063.755
332 «	**TP73 probe** 01682-L24330		1**p**36.32				01-003.558
340 «	**MIR101-1 probe** 13654-L24420		1**p**31.3				01-065.297
347	**TNFRSF14 probe** 04693-L24421		1**p**36.32				01-002.480
355	Reference probe 06426-L05952	6p22					06-024.386
362	**TGFB1 probe** 02889-L23352			19**q**13.2			19-046.542
370	**FUBP1 probe** 18571-L24211		1**p**31.1				01-078.203
377	**DPYD probe** 02870-L23108		1**p**21.3				01-098.159
385	Reference probe 08311-L23302	11q22					11-098.932
391	Reference probe 10464-L23212	2p11					02-085.640
400	**PRDX1 probe** 18410-L23657		1**p**34.1				01-045.749
407 «	**CDKN2C probe** 18566-L24049		1**p**33				01-051.212
413 ¥	**MFN2 probe** 20882-L29180		1**p**36.22				01-011.984
420	**ZNF296 probe** 03221-L24213			19**q**13.32			19-050.271
427 ¬	**CRB1 probe** 06961-L24214		1q31.3				01-195.593
436	Reference probe 10634-L11182	8q12					08-061.856
445 ¥	**PLPP3 probe** 18120-L24277		1**p**32.2				01-056.775
454 ¥	**PARK7 probe** 02189-L29162		1**p**36.23				01-007.968
463 «	**CIC probe** 18575-L24215			19**q**13.2			19-047.487
475	**NRAS probe** 01032-L20220		1**p**13.2				01-115.053
481	Reference probe 09772-L10187	15q21					15-042.706
489	Reference probe 17939-L15290	3q25					03-157.716

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	Chr. 1	Chr. 19	*IDH* mutation	*CDKN2A/B*	Location (hg18) in kb
497	**PRDX1 probe** 18413-L24632	1**p**34.1					01-045.760
504	Reference probe 13438-L24633	5q31					05-131.756

* New in version C2 (from lot C2-0416 onwards).
¥ Changed in version C2 (from lot C2-0416 onwards). Small change in length, no change in sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
« 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.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
¬ 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: Please notify us of any mistakes: info@mlpa.com.

Table 2a. P088-C2 target probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene /Exon	Location/ Ligation site	**Partial** sequence (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
Chromosome 1 Loss of 1p arm is together with loss of 19q arm a diagnostic molecular marker in oligodendrogliomas and it can be used in predicting the response to chemotherapy and prognosis (Smith et al. 2000; Cairncross et al. 1998).						
1**p** arm						
178	02890-L20648	GNB1	1p36.33	CTAAGATCGGAA-GATGAGTGAGCT	733.0 kb	01-001.747
347	04693-L24421	TNFRSF14	1p36.32	CAATACCCTCAT-TCACGGGGAGGA	1.1 **M**b	01-002.480
332 «	01682-L24330	TP73	1p36.32	GAGACCCGGGTG-TCAGGAAAGATG	4.4 **M**b	01-003.558
454	02189-L29162	PARK7	1p36.23	AGAGCAGCGAAC-TGCGACGATCAC	4.0 **M**b	01-007.968
413	20882-L29180	MFN2	1p36.22	CGCAGAAGGCTT-TCAAGTGAGGAT	10.3 **M**b	01-011.984
293 «	06055-L24329	WNT4	1p36.12	GCGAGAAACTCA-AGGGCCTGATCC	6.0 **M**b	01-022.329
163	18115-L23104	PTAFR	1p35.3	TGCCCGCCTGTA-CCCTTGCAAGAA	17.4 **M**b	01-028.350
400	18410-L23657	PRDX1	1p34.1	TACAAACCAGTA-GCCTGCCCACAA	10.9 kb	01-045.749
497	18413-L24632	PRDX1	1p34.1	ACCTCAGCCATC-CGCAACAGGGTG	5.3 **M**b	01-045.760
288	02877-L24219	FAF1	1p33	GGACCTGCATTT-AATCCAGCAAGT	181.6 kb	01-051.026
283 «	18565-L24220	CDKN2C	1p33	CCGGAGGGTTAA-AAGATGATCGCC	4.3 kb	01-051.208
407 «	18566-L24049	CDKN2C	1p33	TGCTGGAGTTTC-AAGCTGATGTTA	5.6 **M**b	01-051.212
445	18120-L24277	PLPP3	1p32.2	AGCACCATCAAG-CCTTACCACCGA	8.5 **M**b	01-056.775
340 «	13654-L24420	MIR101-1	1p31.3	GGATGGCAGCCA-TCTTACCTTCCA	12.9 **M**b	01-065.297
370	18571-L24211	FUBP1	1p31.1	CCATCATGGCGA-TGGACCGGGAAA	10.9 **M**b	01-078.203
259	02871-L19715	GTF2B	1p22.2	CAGATGCGATTT-TAGTGGAGGACT	9.0 **M**b	01-089.126
377	02870-L23108	DPYD	1p21.3	CTGCTGTCACTT-GGCTCTCTGGCT	16.9 **M**b	01-098.159
475	01032-L20220	NRAS	1p13.2	TGATGGGACTCA-GGGTTGTATGGG	5.3 **M**b	01-115.053
131	05745-L05183	NOTCH2	1p12	GGGGTCAACACT-TACAACTGCCGC	34.0 **M**b	01-120.331
1**q** arm						
306 ¬	16877-L19710	LMNA	1q22	ACTGCCTGGCAT-TGTCCAGCTGGA	41.2 **M**b	01-154.372
427 ¬	06961-L24214	CRB1	1q31.3	GGAATGTGTGGA-GCTGTCCTCAGA	4.0 **M**b	01-195.593
196 ¬	06557-L20938	TNNT2	1q32.1	TTTGCTTCCTCT-TCTTCTTCATCT	-	01-199.604
***IDH1*, at 2q33.3** *IDH1* exon numbering according to LRG_610 and indicated ligation sites are in the RefSeq standard NM_005896.3 sequence. The p.R132H (c.395G>A) mutation has been detected by sequencing in 664 samples (92.7%), and the p.R132C (c.394C>T) mutation in 29 samples (4.2%), in a cohort of 1010 WHO grade II and III astrocytomas, oligodendrogliomas and oligoastrocytomas (Hartmann et al. 2009). The *IDH1* mutations described are not activating or inactivating but probably result in an altered enzymatic specificity. The probes at 203 nt and 227 nt will only give a signal when respectively the p.R132H or p.R132C mutations are present in the sample.						
203 §	19529-L16492	IDH1, exon 6; **p. R132H** (c.395G>A)	690-691	CATCATAGGTC**A**-TCATGCTTATGG	0.0 kb	02-208.821
227 §	14787-L23353	IDH1, exon 6; **p.R132C** (c.394C>T)	689-688 (reverse)	ATAAGCATGAC**A**-ACCTATGATGAT	-	02-208.821

Length (nt)	SALSA MLPA probe	Gene /Exon	Location/ Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
***CDKN2A/2B*, at 9p21.3** *CDKN2A* exon numbering according to LRG_11 and ligation sites are in the RefSeq standard NM_058195.3, unless stated otherwise. *CDKN2B* exon numbering and ligation sites as in RefSeq standard NM_078487.2 sequence. Loss of 9p and especially deletions of the 9p21.3 region with *CDKN2A/2B* are common in oligodendroglioma samples (Bigner et al. 1999) and could be used as a marker of poor prognosis (Cairncross et al. 1998). In contrast, *CDKN2A/2B* deletions are rare in anaplastic astrocytomas and glioblastomas with mutated *IDH1* or *IDH2* genes, but are more frequent in these tumours without *IDH1/2* mutations (Yan et al. 2009). Additionally, homozygous deletions of *CDKN2A/2B* have been reported to define a subset of malignant astrocytomas in children (Schiffman et al. 2010).						
153	16881-L23102	CDKN2A, exon 3	45 nt before exon 3	TCCTTTCCGTCA-TGCCGGCCCCCA	3.7 kb	09-021.961
253	16060-L29163	CDKN2A, exon 2	NM_000077.4; 138-139	GCCTGGAAAGAT-ACCGCGGTCCCT	19.4 kb	09-021.965
190	16880-L20211	CDKN2A, exon 1	76-77	AGTCTGCAGTTA-AGGGGGCAGGAG	11.4 kb	09-021.984
232	10337-L23606	CDKN2B, exon 2	1030-1031	GCCTGTCTGAGA-CTCACAGGAAGG	3.1 kb	09-021.996
142	11867-L23298	CDKN2B, exon 1	327-328	CCAACGGTGGAT-TATCCGGGCCGC	-	09-021.999
***IDH2*, at 15q26.1** *IDH2* exon numbering according to LRG_611 and ligation sites are in the RefSeq standard NM_002168.3 sequence. The p.R172K (c.515G>A) mutation has been detected by sequencing in 20 samples (64.5%), and the p.R172M (c.515G>T) mutation in 6 samples (19.3%), in a cohort of 1010 WHO grades II and III astrocytomas, oligodendrogliomas and oligoastrocytomas (Hartmann et al. 2009). The same study suggests that *IDH2* mutations occur predominantly in oligodendroglial tumours. The *IDH2* mutations described are not activating or inactivating but appear to result in an altered enzymatic specificity. The probes at 244 nt and 238 nt will only give a signal when respectively the p.R172M or p.R172K mutations are present in the sample.						
238 §	20963-L29002	IDH2, exon 5; **p.R172K** (c. 515 G>A)	679-680	TACCATTGGCA**A**-GCACGCCCATGG	0.0 kb	15-088.433
244 §	20963-L29001	IDH2, exon 5; **p.R172M** (c.515G>T)	679-680	TACCATTGGCA**T**-GCACGCCCATGG	-	15-088.433
Chromosome 19 Loss of 19q arm is together with loss of 1p arm a diagnostic molecular marker in oligodendrogliomas and it can be used in predicting the response to chemotherapy and prognosis (Smith et al. 2000; Cairncross et al. 1998). The genes FUBP1 (at 1p) and CIC (at 19q) frequently occurred mutated and are suggested to be associated with longer median overall survival (Jiao et al. 2012; Bettegowda et al. 2011).						
19**p** arm						
148 ¬	02488-L22890	SMARCA4	19p13.2	CGTCTTGCAGTC-GGTCTTCACCAG	45.5 kb	19-011.031
214 ¬	02314-L20213	LDLR	19p13.2	TCTGTGACTCAG-ACCGGGACTGCT	23.9 **M**b	19-011.077
19**q** arm						
168 «	02881-L23105	CCNE1	19q12	GATGGTTCCATT-TGCCATGGTTAT	2.8 **M**b	19-035.005
184	02882-L02349	PDCD5	19q13.11	CGAGGAGCTTGA-GGCGCTGAGGAG	3.1 **M**b	19-037.764
157	18116-L23103	UPK1A	19q13.12	GATGGTGTCCAA-CCCATCCCTGAT	4.8 kb	19-040.856
265 Ж «	18117-SP0616-L23106	UPK1A	19q13.12	GTCTCAGGTGTG-27 nt spanning oligo-ATATCCTTAGCC	5.7 **M**b	19-040.861
362	02889-L23352	TGFB1	19q13.2	GAGTGGTTATCT-TTTGATGTCACC	944.1 kb	19-046.542
463 «	18575-L24215	CIC	19q13.2	GAAACATCCTGC-AGACACTGGTGC	2.8 **M**b	19-047.487
420 «	03221-L24213	ZNF296	19q13.32	TCATGGACCACA-AGAAGCTGGGCT	3.8 **M**b	19-050.271
220	02887-L02354	PPP1R15A	19q13.33	GATGTGGATAGT-GAGGATAAGGAA	81.7 kb	19-054.070
300	00348-L00174	BAX	19q13.33	TCCCCCCGAGAG-GTCTTTTTCCGA	9.6 **M**b	19-054.151
326 «	18118-L23300	CHMP2A	19q13.43	TGGAGTTTGAGC-GGCAGGCAGAGA	2.0 kb	19-063.755
313 «	18119-L29136	CHMP2A	19q13.43	GGGCCCTGAACC-GTGCCATGCGGG	-	19-063.757

§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
« 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.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
¬ 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: Exon numbering may differ from literature. When an LRG sequence was available, we have adopted the exon numbering accordingly. Otherwise, the NM sequences indicated in Table 2a are used for exon numbering. Data has been retrieved on 11/2018, but might be changed (e.g. by NCBI) after the release of the product description. Please notify us of any inconsistencies: info@mlpa.com.

Table 2b. Reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
270	16659-L19210	NRXN1	2p16	TAATTTCTGTGG-TTCTTGGGGCTT	02-051.107
391	10464-L23212	GGCX	2p11	CACCATCATGTT-TCTGGGTGAGGG	02-085.640
489	17939-L15290	KCNAB1	3q25	CTTTTCCAGAGA-GAGAAAGTGGAG	03-157.716
319	04833-L22803	NIPBL	5p13	ACGTGTGAAAAT-GAACAAACGCAA	05-037.032
504	13438-L24633	SLC22A5	5q31	GACTTGTATTAT-TTGGCTACAGTC	05-131.756
355	06426-L05952	DCDC2	6p22	TTTAGGGAAATG-ATCGCCACTCTA	06-024.386
436	10634-L11182	CHD7	8q12	GGATCCCAGTAA-AGGTTTTGGTAA	08-061.856
385	08311-L23302	CNTN5	11q22	CACCAGAGCTGT-TAAACACATTGA	11-098.932
173	15449-L23605	COL2A1	12q13	CTGGTATCCTCA-TTTTACTTTTTA	12-046.676
481	09772-L10187	SPG11	15q21	TTTCTTCAGGAT-TGATAGTCATTC	15-042.706
277	17450-L29159	GRIN2A	16p13	TGCAGGATTATA-ATCTCACAATCT	16-009.761
137	09957-L20646	POLR2A	17p13	ACAACAAGAAGA-AGATCATCATCA	17-007.355
122	02844-L02274	NPC1	18q11	GACGAGTCTGTG-GATGAGGTCACA	18-019.394
208	16261-L18553	SAMHD1	20q11	AGTAGACAATGA-GTTGCGTATTTG	20-034.979

Related SALSA MLPA probemixes

- P105 Glioma-2: Contains probes for detection of copy number aberrations of *PDGFRA*, *EGFR*, *CDKN2A*, *PTEN*, *CDK4-MIR26A2-MDM2*, *NFKB1A* and *TP53* genes.
- P370 BRAF-IDH1-IDH2: Contains probes to detect genomic duplications leading to the *KIAA1549-BRAF* and *SRGAP3-RAF1* fusion genes, and identify most common *BRAF*, *IDH1|2* point mutations and copy number alterations of *BRAF*, *CDKN2A/2B*, *FGFR1*, *MYB* and *MYBL1* genes.
- ME012 MGMT-IDH1-IDH2: Contains probes for methylation detection of *MGMT* genes and probes for detection of most common *IDH1|2* point mutations.
- ME024 9p21 CDKN2A/2B region: Contains probes both for methylation and copy number detection for the chromosomal region 9p21 (*CDKN2A/2B*, *MTAP*, *PAX5*).

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P088 Product history	
Version	*Modification*
C2	*Lengths of several probes are adjusted with no change in the sequences detected, one reference probes is replaced and sample DNA used for this probemix is changed from SD021 to SD054.*
C1	*Several target probes and all reference probes are replaced. Also, four probes for point mutations in IDH1 and IDH2, probes for CDKN2A/2B and new control fragments (QDX2) are included.*
B2	*The 88 and 96 nt control fragments have been replaced (QDX2).*
B1	*Several probes have been replaced, incl. three probes for 1q. Extra control fragments have been included.*
A1	*First release.*

Implemented changes in the product description
Version C2-01 – 04 December 2018 (01P) - Product description restructured and adapted to a new template. - For uniformity, the chromosomal positions and bands in this document are now all based on hg18 (NCBI36). - Warning about SNPs removed in Table 1 and 2 for NOTCH2 (05745-L05183), CDKN2B (10337-L23606) and ZNF296 (03221-L24213) probes. *Version 21 – 15 August 2017 (T08)* - One new reference for P088 added on page 1. - Small layout changes and corrections of typos in the document. *Version 20 – 2 May 2016 (T08)* - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included). - A new SD is available for P088-C2-0416. The previous SD021 is changed to SD054. - New references added on pages 1 and 2. - Warning added in Table 1, SNP rs531705888 could influence the probe signal at probe 232nt 10337-L23606. - PLPP3 gene name has been changed (PPAP2B previously) in Table 1 and 2. *Version 19 – 22 May 2015 (T07)* - Information on the p.R172M (G515T) mutation corrected in Table 2. - Minor restructuring of the document.

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