Product Description SALSA® MLPA® Probemix P181-B2 Centromere mix 1 – P182-B2 Centromere mix 2

To be used with the MLPA General Protocol.

P181 Version B2. P182 Version B2. For complete product history see page 9.

Catalogue numbers:
- **P181-025R:** SALSA MLPA Probemix P181 Centromere mix 1, 25 reactions.
- **P182-025R:** SALSA MLPA Probemix P182 Centromere mix 2, 25 reactions.
- **P181-050R:** SALSA MLPA Probemix P181 Centromere mix 1, 50 reactions.
- **P182-050R:** SALSA MLPA Probemix P182 Centromere mix 2, 50 reactions.
- **P181-100R:** SALSA MLPA Probemix P181 Centromere mix 1, 100 reactions.
- **P182-100R:** SALSA MLPA Probemix P182 Centromere mix 2, 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 Probemixes P181 Centromere mix 1 and P182 Centromere mix 2 are **research use only (RUO)** assays for the detection of deletions or duplications in genes close to the centromeres of all chromosomes, with the exception of the Y-chromosome. In most cases, probes are included for the first well-characterised gene in the centromeric region. Possible applications of these probemixes are in cancer research, as well as for characterisation of marker chromosomes.

These SALSA MLPA Probemixes are not CE/FDA registered for use in diagnostic procedures. Purchase of these products 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 P181-B2 Centromere mix 1 contains 46 MLPA probes with amplification products between 130 and 450 nucleotides (nt). The SALSA MLPA Probemix P182-B2 Centromere mix 2 contains 46 MLPA probes with amplification products between 131 and 453 nt. Both probemixes include one probe for each of the chromosome arms (except the Y-chromosome). For the acrocentric chromosomes (13, 14, 15, 21 and 22), which have more than 10 Mb of repeat sequences at one end covering most or all of the p-arms, there are two probes on the q-arm in each probemix, close to the centromere. Probes in P181-B1 and P182-B2 detect different sequences. Complete probe sequences are available online (www.mlpa.com).

The P181-B2 Probemix contains ten quality control fragments generating amplification products between 64 and 121 nt: four DNA Quantity Fragments (Q-fragments), two DNA Denaturation Fragments (D-fragments), one Benchmark Fragment, one chromosome X and two chromosome Y-specific fragments (see table below).

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)
105-121	Y-fragments (Y chromosome specific)

The P182-B2 Probemix contains ten quality control fragments generating amplification products between 64 and 118 nt: four DNA Quantity Fragments (Q-fragments), two DNA Denaturation Fragments (D-fragments), one Benchmark Fragment, one chromosome X and two chromosome Y-specific fragments (see table below).

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)
105-118	Y-fragments (Y chromosome specific)

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.

No DNA controls result in only 5 major peaks shorter than 121 nt: 4 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 4 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, 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.

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, healthy, individuals. 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 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. 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. 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 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.

Please note that these above mentioned dosage quotients are not applicable to the X-chromosome specific probes. Deletions of the recognition sequences of X-chromosome specific probes will lead to a complete absence of the corresponding probe amplification product in males, whereas female heterozygotes are recognisable by a 35-60% reduction in relative peak height.

- 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.

Limitations of the procedure:
- 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.

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.

Please report false positive results due to SNPs and other unusual results to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P181-B2 Centromere mix 1

Length (nt)	Chromosomal position (hg18)	Gene detected	SALSA MLPA probe	Location (hg18) in kb
64-121 ¥	Control fragments – see table in probemix content section for more information			
130 +	1q21.1	PDE4DIP	05712-L05712	01-143.658 +
137	2q11.1	MAL	05714-L05152	02-095.077
143	3q11.2	PROS1	05294-L06017	03-095.112
148 «	4q12	SGCB	05907-L02768	04-052.589
154	5q11.2	ISL1	20445-L27929	05-050.723
160	6q12	PTP4A1	05719-L05158	06-064.348
166	7q11.21	GUSB	05721-L05160	07-065.063
172 «	8q11.21	KIAA0146	06239-L05745	08-048.810
178	9q21.11	TJP2	05725-L05164	09-071.041
184 «	10q11.21	RET	05908-L04937	10-042.945
190	11q12.1	APLNR (AGTRL1)	05727-L05166	11-056.758
196	12q12	KIF21A	05728-L05167	12-038.050
202	13q12.11	ZMYM2 (ZNF198)	05730-L06767	13-019.466
208	14q11.2	APEX1	05731-L05170	14-019.995
214 «	15q11.2	NDN	06282-L01542	15-021.482
220 «	16q11.2	ORC6	05735-L05174	16-045.289
226	17q11.1	WSB1	05736-L05175	17-022.663
233	18q11.1	ROCK1	05737-L05176	18-016.840
240	19q12	POP4	06211-L05178	19-034.798
247 «	20q11.21	DUSP15	06240-L05746	20-029.919
254	21q11.2	SAMSN1	05911-L05356	21-014.811
261 «	22q11.1	CECR5	05742-L05180	22-016.011
267	**X**q11.1	ZC4H2 (HCA127)	05744-L05182	**X**-064.113
274	1p12	NOTCH2	06241-L05747	01-120.314
283	2p11.2	RPIA	05713-L05151	02-088.779
290	3p11.1	EPHA3	06498-L06038	03-089.342
297	4p12	OCIAD1	05716-L05155	04-048.549
305	5p12	FGF10	06212-L05156	05-044.341
312	6p12.1	RAB23	05718-L05157	06-057.180
319 «	7p11.2	GBAS	06242-L05748	07-056.013
330	8p11.21	FNTA	05722-L05161	08-043.052
337	9p13.1	IGFBPL1	05724-L05163	09-038.399
346	10p11.21	ZNF25	06214-L06020	10-038.283
355	11p11.2	PTPRJ	05912-L27746	11-048.102
364	12p11.21	PKP2	06411-L27747	12-032.895
371	16p11.2	TGFB1I1	06216-L13376	16-031.393
378 +	17p11.2	MAP2K3	05913-L05715	17-021.159 +
385	18p11.21	RNMT	05914-L05359	18-013.724
394 «	19p13.11	ATP13A1	20192-L16586	19-019.629
402	20p11.21	PYGB	05740-L05179	20-025.198
409	**X**p11.1	UBQLN2	05743-L05181	**X**-056.608
418	13q12.11	MPHOSPH8 (HSMPP8)	09672-L05168	13-019.131
425	14q11.2	PARP2 (ADPRTL2)	05915-L05360	14-019.894
433 ~	15q11.2	NIPA2	05732-L05171	15-020.566
441	21q11.2	HSPA13 (STCH)	05916-L05361	21-014.668
450	22q11.1	CECR1	05741-L05219	22-016.070

¥ Changed in version B2 (from lot B2-0417 onwards). Small change in length, 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.
+ These probes detect a second target site on 1**p**11.2 (PDE4DIP, 130 nt probe), or on 17p11.2 (MAP2K3, 378 nt probe). These second target sites are only present in the hg38 genome build but not in the hg18/hg19 builds. Inconclusive results for these probes can be verified by using the P182 Centromere mix-2.
~ This 15q NIPA2 probe has been reported to be deleted in healthy individuals. Some healthy individuals have only one copy of the 15q NIPA1, NIPA2, CYFIP1 and TUB genes.

Table 1b. SALSA MLPA Probemix P182-B2 Centromere mix 2

Length (nt)	Chromosomal position (hg18)	Gene detected	SALSA MLPA probe	Location (hg18) in kb
64-118 ¥	Control fragments – see table in probemix content section for more information			
131	1p12	NOTCH2	05745-L05183	01-120.331
136	2p11.2	EIF2AK3	05747-L05185	02-088.674
142	3p11.2	EPHA3	05917-L05362	03-089.539
147 «	4p12	ZAR1	15147-L16921	04-048.191
154	5p12	HCN1	05751-L05189	05-045.498
160 +	6p11.2	PRIM2	06497-L06037	06-057.355 +
166	7p11.2	SUMF2	05755-L06276	07-056.110
172	8p11.21	HOOK3	06244-L06278	08-042.982
178	9p13.2	EXOSC3	05758-L05196	09-037.771
184	10p11.21	ZNF25	05760-L06666	10-038.286
190	11p11.2	PTPRJ	05918-L05363	11-048.106
196 «	12p11.21	PKP2	05655-L04781	12-032.941
202	13q12.11	MPHOSPH8 (HSMPP8)	05763-L05201	13-019.141
208	14q11.2	PARP2 (ADPRTL2)	05765-L05203	14-019.895
214 «	15q11.2	MAGEL2	15148-L11839	15-021.440
220	16p11.2	AHSP (ERAF)	05769-L05207	16-031.447
226	17p11.2	USP22	15144-L16919	17-020.865
232	18p11.21	RNMT	05772-L05210	18-013.732
240 «	19p13.11	GMIP	05774-L05212	19-019.602
247	20p11.21	ZNF337	05775-L05720	20-025.615
255	21q11.2	HSPA13 (STCH)	05919-L05364	21-014.666
263	22q11.1	CECR1	05777-L10758	22-016.068
269 ¥	**X**p11.21	MAGEH1	05779-L28310	**X**-055.496
276	1q21.1	HFE2	05746-L05184	01-144.128
283	2q11.1	ZNF2	05748-L05186	02-095.210
289	3q11.2	PROS1	06238-L04687	03-095.095
298	4q12	USP46	05750-L05188	04-053.189
305	5q11.2	ISL1	05752-L05190	05-050.716
311	6q11.1	KHDRBS2	06246-L05752	06-062.816
319	7q11.21	ASL	06805-L06400	07-065.192
330	8q11.21	PRKDC	05757-L05195	08-049.029
337	9q21.11	TJP2	05759-L06281	09-071.059
346 ¥	10q11.21	RET	06218-L28309	10-042.934
353	11q12.1	APLNR (AGTRL1)	05761-L05721	11-056.761
360	12q12	KIF21A	05762-L05200	12-037.975
369	16q11.2	VPS35	05770-L05208	16-045.260
378	17q11.1	WSB1	05921-L05366	17-022.663
386	18q11.2	MIB1	05773-L05211	18-017.678
393 «	19q12	CCNE1	15145-L17579	19-035.006
400 «	20q11.21	REM1	05781-L01485	20-029.528
409	**X**q11.1	ARHGEF9	05780-L05218	**X**-062.792
418	13q12.11	ZMYM2 (ZNF198)	05764-L05202	13-019.506
427	14q11.2	APEX1	06493-L17676	14-019.995
433	15q11.2	MKRN3	06219-L01539	15-021.363
441	21q11.2	SAMSN1	05776-L05723	21-014.815
453	22q11.21	SLC25A18	06220-L04860	22-016.423

¥ Changed in version B2 (from lot B2-0115 onwards). Small change in length, 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.

+ This probe detects a second target site on 6**q**11.1 (PRIM2, 160 nt probe). This second target site is only present in the hg38 genome build but not in the hg18/hg19 builds. Inconclusive results for this probe can be verified by using the P181 Centromere mix-1.

Table 2. P181/P182 probes arranged according to chromosomal location

Length (nt)		SALSA MLPA probe	Gene	Location	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
P181	P182						
274		06241-L05747	NOTCH2	1p12	CTGTGTATGTGT-CAACGGCTGGAG	17 kb	01-120.314
	131	05745-L05183	NOTCH2	1p12	GGGGTCAACACT-TACAACTGCCGC		01-120.331
130 +		05712-L05712	PDE4DIP	1q21.1	GCTACATCTGTT-GGAGGAGCCAAC	470 kb	01-143.658 +
	276	05746-L05184	HFE2	1q21.1	GCTGAGGTGGAT-AATCTTCCTGTA		01-144.128
	136	05747-L05185	EIF2AK3	2p11.2	TAGTGACGAAAT-GGAACAAGAGGA	105 kb	02-088.674
283 #		05713-L05151	RPIA	2p11.2	TGGTTCTACAAT-TGTCCATGCTGT		02-088.779
137		05714-L05152	MAL	2q11.1	CGTGTCTGTGTT-CTGCTTCGTGGC	132 kb	02-095.077
	283	05748-L05186	ZNF2	2q11.1	CTGATGTGATTT-TCCAATTGAAGA		02-095.210
290		06498-L06038	EPHA3	3p11.2	GAGAGTATACTT-CAAAAAGTGCCC	197 kb	03-089.342
	142	05917-L05362	EPHA3	3p11.1	CCAAGTGGTCAT-GATCGCCATTTC		03-089.539
	289 #	06238-L04687	PROS1	3q11.2	ATAGATTCTGCG-TACAGTATCACG	18 kb	03-095.095
143 #		05294-L06017	PROS1	3q11.2	CTGCTGCACGTC-AGTCAACTAATG		03-095.112
	147 «	15147-L16921	ZAR1	4p12	ACTTCGCCACGT-GGACCCTAAACG	358 kb	04-048.191
297		05716-L05155	OCIAD1	4p12	ATGCTTCCTCAT-TATGAGCCAATT		04-048.549
148 «		05907-L02768	SGCB	4q12	TGTATTCATTAT-GGGCAAAACCAT	600 kb	04-052.589
	298	05750-L05188	USP46	4q12	CAGCAAAAGAAG-AAGGAAAACTTG		04-053.189
305		06212-L05156	FGF10	5p12	AGGATAGAGGAA-AATGGATACAAT	1157 kb	05-044.341
	154	05751-L05189	HCN1	5p12	GTCTTCAGTTCT-TAGTACCACTAC		05-045.498
	305	05752-L05190	ISL1	5q11.2	GGCAATCAGATT-CACGATCAGTAT	7 kb	05-050.716
154		20445-L27929	ISL1	5q11.2	TGGAAGTACAAA-GTTACCAGCCAC		05-050.723
312		05718-L05157	RAB23	6p12.1	AGGTCAGGAGGA-ATTTGATGCAAT	174 kb	06-057.180
	160 +	06497-L06037	PRIM2	6p11.2	GCAATCATCCTG-AATGAATTTAGA		06-057.355 +
	311	06246-L05752	KHDRBS2	6q11.1	CAAGAGGAAACT-CCTTGAAGAGGC	1532 kb	06-062.816
160 #		05719-L05158	PTP4A1	6q12	GCAACCTCTGTA-TTTGGGTTACAG		06-064.348
319 «		06242-L05748	GBAS	7p11.2	CAACAGATCTCG-AGAAGACAGCTG	96 kb	07-056.013
	166	05755-L06276	SUMF2	7p11.2	GCATCCGAGAGA-GACTGGAGCACC		07-056.110
166		05721-L05160	GUSB	7q11.21	CTTCACTCGGCA-GAGACAACCAAA	129 kb	07-065.063
	319	06805-L06400	ASL	7q11.21	TTTGAAGTGTCA-GACACTATGAGT		07-065.192
	172	06244-L06278	HOOK3	8p11.21	TAATGAACTACA-GAAGAAGAGAGC	69 kb	08-042.982
330 #		05722-L05161	FNTA	8p11.21	GACCAACTTCTG-AAAGAGGATGTG		08-043.052
172 «		06239-L05745	KIAA0146	8q11.21	GGGTTGTTAAAT-TGTTTTGTCCAG	218 kb	08-048.810
	330	05757-L05195	PRKDC	8q11.21	GGTGAAGTTCAT-CCTAGTGAGATG		08-049.029
	178	05758-L05196	EXOSC3	9p13.2	GAAAACAGATCT-TCTCCAGATTGG	628 kb	09-037.771
337		05724-L05163	IGFBPL1	9p13.1	GTCAAATAACGG-ATCTTTGTGCTT		09-038.399
178		05725-L05164	TJP2	9q21.11	CGTTTTTTATAA-GAAGCCACTTTG	18 kb	09-071.041
	337	05759-L06281	TJP2	9q21.11	ATGCACCATGGA-GACGTGGTGGGA		09-071.059
346		06214-L06020	ZNF25	10p11.21	TCTAGAAGCAAG-ATACCAGGAAAG	4 kb	10-038.283
	184	05760-L06666	ZNF25	10p11.21	ATGTTATTGTGG-AATTCACCAAGG		10-038.286
	346 ¥	06218-L28309	RET	10q11.21	CCTGCTGTCAGA-GTTCAACGTCCT	11 kb	10-042.934
184 «		05908-L04937	RET	10q11.21	CCCAGAATTGCT-GACAGCAGAGGC		10-042.945
355		05912-L27746	PTPRJ	11p11.2	GGGTTCTTCTTG-AAAGCATTGGAA	4 kb	11-048.102
	190	05918-L05363	PTPRJ	11p11.2	GGGGAGACAGAT-TCTTCCAATCTC		11-048.106
190		05727-L05166	APLNR »	11q12.1	CCAGTGCCTTCT-TCAGAATATCTG	4 kb	11-056.758
	353	05761-L05721	APLNR »	11q12.1	GGGGTAAGGCAA-GAGAGGGTGGAG		11-056.761
364 #		06411-L27747	PKP2	12p11.21	TGAGAAACTTAG-TATTTGAAGACA	46 kb	12-032.895
	196 «	05655-L04781	PKP2	12p11.21	TCCGGACCGTCC-TGGGCCAGCAGA		12-032.941
	360	05762-L05200	KIF21A	12q12	AGGCTCGCAATT-TGCAAGATGGTC	76 kb	12-037.975
196		05728-L05167	KIF21A	12q12	AAAGATAAGGCT-TTTACTTTTGAC		12-038.050
418		09672-L05168	MPHOSPH8»	13q12.11	AAGTTGGAAGAT-TTCCAAAAGCAC	9 kb	13-019.131
	202	05763-L05201	MPHOSPH8»	13q12.11	CTAGAACCAGTT-TTTCCAATCGCA	325 kb	13-019.141
202		05730-L06767	ZMYM2 »	13q12.11	AGACTCTGAAAT-TCAGATTGCTAA	41 kb	13-019.466
	418	05764-L05202	ZMYM2 »	13q12.11	ACTTGTTCAGAT-GACTATAAGAAG		13-019.506
425		05915-L05360	PARP2 »	14q11.2	CAATCTACCCAT-GCTCCCACACAC	1 kb	14-019.894
	208	05765-L05203	PARP2 »	14q11.2	CTCTCGCCTAAA-GAATACAGGACT	100 kb	14-019.895
208		05731-L05170	APEX1	14q11.2	ACCAAATGTTCA-GAGAACAAACTA	1 kb	14-019.995
	427	06493-L17676	APEX1	14q11.2	AGACCTCAATGT-GGCACATGAAGA		14-019.995
433 ~		05732-L05171	NIPA2	15q11.2	GTGGCCAACTTC-GCTGCGTATGCG	797 kb	15-020.566
	433	06219-L01539	MKRN3	15q11.2	GGCTGCAGACCT-TGCACCCCATGG	78 kb	15-021.363
	214 «	15148-L11839	MAGEL2	15q11.2	AGCAAGATGCTT-GTCCTGAGGTTT	42 kb	15-021.440
214 «		06282-L01542	NDN	15q11.2	ACACTGCTGCGA-GGGTAGTGGGCA		15-021.482
371		06216-L13376	TGFB1I1	16p11.2	CAGGAACTTAAT-GCCACTCAGTTC	54 kb	16-031.393
	220	05769-L05207	AHSP »	16p11.2	GTTCAGCGTTCT-GCTGAATCAGCA		16-031.447
	369	05770-L05208	VPS35	16q11.2	CCTTTGGTATTT-GCAGCTTACCAG	29 kb	16-045.260

Length (nt) P181	P182	SALSA MLPA probe	Gene	Location	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
220 «		05735-L05174	ORC6	16q11.2	AAACCACAGAAA-GATGAAGATCTG		16-045.289
	226	15144-L16919	USP22	17p11.2	CCTTGGCGATTA-TTTCCATGTCTT	294 kb	17-020.865
378 +		05913-L05715	MAP2K3	17p11.2	ACTTTGCACACT-TTGGCCCAGGGT		17-021.159 +
226		05736-L05175	WSB1	17q11.1	ATTGATGAGGAT-TATCCAGTGCAA	1 kb	17-022.663
	378	05921-L05366	WSB1	17q11.1	TGTCAATCCGAA-GAGTGATGCCCA		17-022.663
385		05914-L05359	RNMT	18p11.21	TACAATGAACTT-CAGGAAGTTGGT	7 kb	18-013.724
	232	05772-L05210	RNMT	18p11.21	ATGCTGAGAAAT-GCGTGTGAGAGA		18-013.732
233		05737-L05176	ROCK1	18q11.1	AGATGAGCAAGT-CAATTAGTCAGT	838 kb	18-016.840
	386	05773-L05211	MIB1	18q11.2	GGCATCACACTT-TGTCTCAGCTAC		18-017.678
	240 «	05774-L05212	GMIP	19p13.11	TAAGGAAAAACT-ATTTAATACATG	27 kb	19-019.602
394 «		20192-L16586	ATP13A1	19p13.11	CTACAGCGTCTT-TACGCTATCCAT		19-019.629
240		06211-L05178	POP4	19q12	CGATGGCTTTAT-TTCCTACATTTA	208 kb	19-034.798
	393 «	15145-L17579	CCNE1	19q12	TTGTCTGAACAA-AATAGGGCTTCT		19-035.006
402		05740-L05179	PYGB	20p11.21	GAGATAGAAGAA-GATGCTGGCCTT	417 kb	20-025.198
	247	05775-L05720	ZNF337	20p11.21	TTGGGGATGTCA-CTGTGGATTTCA		20-025.615
	400 «	05781-L01485	REM1	20q11.21	GGGATCTGGAAG-AAGCCATACAGC	391 kb	20-029.528
247 «		06240-L05746	DUSP15	20q11.21	GATCACACACAT-CATCTCTATCCA		20-029.919
	255	05919-L05364	HSPA13 »	21q11.2	GCTTTTGGAAAC-AATTGACAAAAT	2 kb	21-014.666
441		05916-L05361	HSPA13 »	21q11.2	ATTCAGCAAGTA-TTGAAAGAAGGC	143 kb	21-014.668
254		05911-L05356	SAMSN1	21q11.2	CCCACAAATGGA-AGTGGAGAACAA	4 kb	21-014.811
	441	05776-L05723	SAMSN1	21q11.2	ATTTCGATCGTT-TTCGGAATAATT		21-014.815
261 «		05742-L05180	CECR5	22q11.1	CTCTGAAAGCCT-TCCGAAGGCTGG	58 kb	22-016.011
	263	05777-L10758	CECR1	22q11.1	CTGGTGAGGAAT-GTCACCTACAGG	2 kb	22-016.068
450		05741-L05219	CECR1	22q11.1	GACGCTCAAAAT-CGCTGAGATGAA	353 kb	22-016.070
	453	06220-L04860	SLC25A18	22q11.21	GCAGTGAGAAGA-GTCGAGTGAAGC		22-016.423
	269 ¥	05779-L28310	MAGEH1	Xp11.21	CAAACTGAAAGT-CATGCATTTTGT	1112 kb	X-055.496
409		05743-L05181	UBQLN2	Xp11.1	AGACACTCGAAA-TTGCCAGGAATC		X-056.608
	409	05780-L05218	ARHGEF9	Xq11.1	ACATCTGTTCTT-TGCCAAGAAGCT	1321 kb	X-062.792
267		05744-L05182	ZC4H2 »	Xq11.1	CATGTGCAAATT-GGAAAGCATTAA		X-064.113
	118 ¥	*S0135-L13810*	*ZFY*	*Yp11.31*			*Y-002.889*
121 ¥		*S0135-L27687*	*ZFY*	*Yp11.31*			*Y-002.889*

¥ Changed in version B2 (from lot B2-0417 onwards). Small change in length, 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.

» These genes are also known as: APLNR (AGTRL1); MPHOSPH8 (HSMPP8); ZMYM2 (ZNF198); PARP2 (ADPRTL2); AHSP (ERAF); HSPA13 (STCH); ZC4H2 (HCA127).

~ This 15q NIPA2 probe has been reported to be deleted in healthy individuals. Some healthy individuals have only one copy of the 15q NIPA1, NIPA2, CYFIP1 and TUB genes.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

+ These probes detect a second target site on 1**p**11.2 (PDE4DIP, 130 nt probe), on 6**q**11.1 (PRIM2, 160 nt probe), or on 17p11.2 (MAP2K3, 378 nt probe). These second target sites are only present in the hg38 genome build but not in the hg18/hg19 builds. Inconclusive results for these probes can be verified by using the other Centromere mix (P182 as confirmation for the PDE4DIP and MAP2K3 probes, P181 as confirmation for the PRIM2 probe).

References

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- Martínez JG et al (2012) Localization of centromeric breaks in head and neck squamous cell carcinoma. *Cancer Genet.* 205:622-9.
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- van Opstal D et al (2011) Multiplex ligation dependent probe amplification (MLPA) for rapid distinction between unique sequence positive and negative marker chromosomes in prenatal diagnosis. *Molecular Cytogenetics.* 14;4:2.
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P181 Product history	
Version	*Modification*
B2	The 118 nt Y-probe has been elongated to 121 nt.
B1	One probe and two denaturation control fragments (88 and 96 nt, QDX2) have been replaced.
A2	Four extra control fragments have been added. Two probes have a small change in length but no change in sequence detected.
A1	First release.

P182 Product history	
Version	*Modification*
B2	Small change in sequence of three LPOs; the 88 and 96 nt DNA denaturation control probes have been replaced (QDX2).
B1	Four probes have been replaced and four extra control fragments have been added.
A1	First release.

Implemented changes in the product description
Version B2-01 - 18 January 2019 (01P) - Product description restructured and adapted to a new template. - Various minor textual or layout changes. - Additional information on second target site for PDE4DIP, MAP2K3 and PRIM2 probes added to Table 1 and 2. - For uniformity, the chromosomal positions and bands in this document are now all based on hg18

(NCBI36).
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 17 - 24 October 2017 (55)
- 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).

Version 16 (54) - 2 March 2015
- 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).

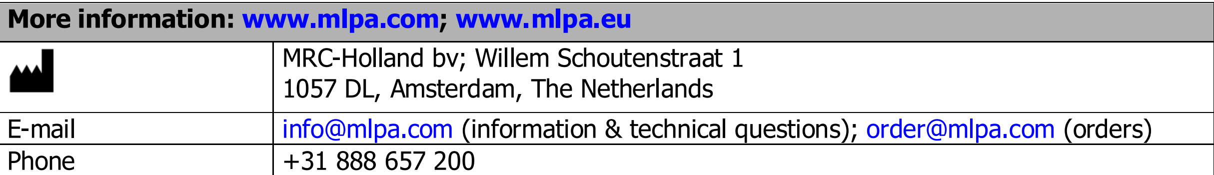
Version 15 (54) - 14 January 2015
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).

Version 14 (53)
- New references added on page 1 and several typos corrected.

Version 13 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 12 (48)
- Small correction of chromosomal locations in Table 1 and 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various minor textual changes.

Version 11 (45)
- 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).
- Textual changes on page 1. Data analysis section has been modified. Tables have been numbered.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.



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