Product Description
SALSA® MLPA® Probemix P181-C1 Centromere mix 1

To be used with the MLPA General Protocol.

Version C1
As compared to version B2, four probes changed in length but not in sequence detected and 19 probes were replaced. For complete product history see page 8.

Catalogue numbers:
- **P181-025R:** SALSA MLPA Probemix P181 Centromere mix 1, 25 reactions.
- **P181-050R:** SALSA MLPA Probemix P181 Centromere mix 1, 50 reactions.
- **P181-100R:** SALSA MLPA Probemix P181 Centromere mix 1, 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 P181 Centromere mix 1 is a **research use only (RUO)** assay 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 this probemix are in cancer research, as well as for characterisation of marker chromosomes and the detection of aneuploidies.

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 P181-C1 Centromere mix 1 contains 46 MLPA probes with amplification products between 127 and 450 nucleotides (nt). This includes 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, close to the centromere. The SALSA MLPA Probemix P182 Centromere mix 2 detects different sequences in the same regions. Complete probe sequences are available online (www.mrcholland.com).

This 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, and one chromosome X and two chromosome Y-specific fragments (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)
105-121	Y-fragments (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).

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, 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 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 (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. 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 final ratio (FR) of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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 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. 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. 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.
- 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.

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. 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.

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.

It is recommended that results of P181 Centromere Mix 1 are confirmed with P182 Centromere Mix 2. All P181 probes differ from P182 probes.

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 SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P181-C1 Centromere mix 1

Length (nt)	SALSA MLPA probe	Gene detected	Chromosomal position (hg18)	Location (hg18) in kb
64-121	Control fragments – see table in probemix content section for more information			
127 *	22652-L31868	*CD160*	1q21.1	01-144,415
137	05714-L05152	*MAL*	2q11.1	02-095,077
142 *	23025-L32476	*PROS1*	3q11.2	03-095,108
148 «	05907-L02768	*SGCB*	4q12	04-052,589
154	20445-L27929	*ISL1*	5q11.2	05-050,723
162 *	23026-L32477	*PTP4A1*	6q12	06-064,346
167 ¥	05721-L32621	*GUSB*	7q11.21	07-065,063
172 «	06239-L05745	*SPIDR (KIAA0146)*	8q11.21	08-048,810
178 *	23027-L32478	*TJP2*	9q21.11	09-071,042
184 ¥ «	23005-L32434	*RET*	10q11.21	10-042,945
193 ¥	05727-L32435	*APLNR*	11q12.1	11-056,758
199 *	23028-L32724	*KIF21A*	12q12	12-038,047
203 *	23029-L32480	*ZMYM2*	13q12.11	13-019,492
208 ¥	05731-L27148	*APEX1*	14q11.2	14-019,995
213 *	23030-L32481	*TUBGCP5*	15q11.2	15-020,394
220	05735-L05174	*ORC6*	16q11.2	16-045,289
226	05736-L05175	*WSB1*	17q11.1	17-022,663
233	05737-L05176	*ROCK1*	18q11.1	18-016,840
240	06211-L05178	*POP4*	19q12	19-034,798
247	06240-L05746	*DUSP15*	20q11.21	20-029,919
254	05911-L05356	*SAMSN1*	21q11.2	21-014,811
261 «	05742-L05180	*HDHD5 (CECR5)*	22q11.1	22-016,011
268 *	23031-L32482	*ZC4H2*	Xq11.1	**X**-064,057
274 *	23032-L32679	*NOTCH2*	1p12	01-120,312
283 *	23033-L32484	*RPIA*	2p11.2	02-088,810
290	06498-L06038	*EPHA3*	3p11.2	03-089,342
297	05716-L05155	*OCIAD1*	4p12	04-048,549
301 *	23034-L32485	*FGF10*	5p12	05-044,346
312 *	23035-L32486	*RAB23*	6p12.1	06-057,163
319 *	23037-L32488	*NIPSNAP2 (GBAS)*	7p11.2	07-056,013
329 *	23038-L32489	*POMK*	8p11.21	08-043,077
337 *	23039-L32490	*IGFBPL1*	9p13.1	09-038,401
346	06214-L06020	*ZNF25*	10p11.21	10-038,283
355	05912-L27746	*PTPRJ*	11p11.2	11-048,102
364 *	23042-L32622	*PKP2*	12p11.21	12-032,922
371	06216-L13376	*TGFB1I1*	16p11.2	16-031,393
379 *	22643-L31855	*TMEM11*	17p11.2	17-021,043
385	05914-L05359	*RNMT*	18p11.21	18-013,724
394 «	20192-L16586	*ATP13A1*	19p13.11	19-019,629
401 *	23040-L32623	*NINL*	20p11.21	20-025,441
409	05743-L05181	*UBQLN2*	Xp11.1	**X**-056,608
418	09672-L05168	*MPHOSPH8*	13q12.11	13-019,131
425	05915-L05360	*PARP2*	14q11.2	14-019,894
432 *	23041-L32492	*MKRN3*	15q11.2	15-021,363
441	05916-L05361	*HSPA13*	21q11.2	21-014,668
450	05741-L05219	*ADA2 (CECR1)*	22q11.1	22-016,070

* New in version C1.
¥ Changed in version C1. 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.

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. P181-C1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal position (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
274	23032-L32679	*NOTCH2*	1p12	ATCTTATCCAGA-CAGGTAGCATCA	01-120,312
127	22652-L31868	*CD160*	1q21.1	CCATAAGCCAAG-TCACACCGTTGC	01-144,415
283	23033-L32484	*RPIA*	2p11.2	CTTGACCCTCAG-TGATCTGGATCG	02-088,810
137	05714-L05152	*MAL*	2q11.1	CGTGTCTGTGTT-CTGCTTCGTGGC	02-095,077
290	06498-L06038	*EPHA3*	3p11.2	GAGAGTATACTT-CAAAAAGTGCCC	03-089,342
142 #	23025-L32476	*PROS1*	3q11.2	GCCATGCAATGA-AGATGGATATAT	03-095,108
297	05716-L05155	*OCIAD1*	4p12	ATGCTTCCTCAT-TATGAGCCAATT	04-048,549
148 «	05907-L02768	*SGCB*	4q12	TGTATTCATTAT-GGGCAAAACCAT	04-052,589
301	23034-L32485	*FGF10*	5p12	CCGTCAAAGCCA-TTAACAGCAACT	05-044,346
154	20445-L27929	*ISL1*	5q11.2	TGGAAGTACAAA-GTTACCAGCCAC	05-050,723
312	23035-L32486	*RAB23*	6p12.1	TAGCAGCTGTAG-CATACCCTAAGA	06-057,163
162	23026-L32477	*PTP4A1*	6q12	GTTCTTGTAAGT-ATTTAACAGTTC	06-064,346
319	23037-L32488	*NIPSNAP2* »	7p11.2	AGATCTCGAGAA-GACAGCTGGCTA	07-056,013
167	05721-L32621	*GUSB*	7q11.21	CTTCACTCGGCA-GAGACAACCAAA	07-065,063
329	23038-L32489	*POMK*	8p11.21	ACCTGAGCTGGA-GAAGGAGATGCG	08-043,077
172 «	06239-L05745	*SPIDR* »	8q11.21	GGGTTGTTAAAT-TGTTTTGTCCAG	08-048,810
337	23039-L32490	*IGFBPL1*	9p13.1	GTGACGGTTCTA-GATCTGAGTAAA	09-038,401
178	23027-L32478	*TJP2*	9q21.11	TCGGGAAGACCT-CACAGCTGTTGT	09-071,042
346	06214-L06020	*ZNF25*	10p11.21	TCTAGAAGCAAG-ATACCAGGAAAG	10-038,283
184 «	23005-L32434	*RET*	10q11.21	CCCAGAATTGCT-GACAGCAGAGGC	10-042,945
355	05912-L27746	*PTPRJ*	11p11.2	GGGTTCTTCTTG-AAAGCATTGGAA	11-048,102
193	05727-L32435	*APLNR*	11q12.1	CCAGTGCCTTCT-TCAGAATATCTG	11-056,758
364	23042-L32622	*PKP2*	12p11.21	CACTTTGACACA-TACCACAGACAG	12-032,922
199	23028-L32724	*KIF21A*	12q12	CGAGAGCTCTGA-ACATTCATCTGG	12-038,047
418	09672-L05168	*MPHOSPH8*	13q12.11	AAGTTGGAAGAT-TTCCAAAAGCAC	13-019,131
203	23029-L32480	*ZMYM2*	13q12.11	TCCTGAAGGAGG-TTCGAGATCACA	13-019,492
425	05915-L05360	*PARP2*	14q11.2	CAATCTACCCAT-GCTCCCACACAC	14-019,894
208	05731-L27148	*APEX1*	14q11.2	ACCAAATGTTCA-GAGAACAAACTA	14-019,995
213	23030-L32481	*TUBGCP5*	15q11.2	CCGTTAGAAGAA-CAAGATCAAAAC	15-020,394
432	23041-L32492	*MKRN3*	15q11.2	ATGCTCTATAAA-AGCATTAAGAAG	15-021,363
371	06216-L13376	*TGFB1I1*	16p11.2	CAGGAACTTAAT-GCCACTCAGTTC	16-031,393
220	05735-L05174	*ORC6*	16q11.2	AAACCACAGAAA-GATGAAGATCTG	16-045,289
379	22643-L31855	*TMEM11*	17p11.2	TCTCATGCACAA-TGTAGCAGTCTG	17-021,043
226	05736-L05175	*WSB1*	17q11.1	ATTGATGAGGAT-TATCCAGTGCAA	17-022,663
385	05914-L05359	*RNMT*	18p11.21	TACAATGAACTT-CAGGAAGTTGGT	18-013,724
233	05737-L05176	*ROCK1*	18q11.1	AGATGAGCAAGT-CAATTAGTCAGT	18-016,840
394 «	20192-L16586	*ATP13A1*	19p13.11	CTACAGCGTCTT-TACGCTATCCAT	19-019,629
240	06211-L05178	*POP4*	19q12	CGATGGCTTTAT-TTCCTACATTTA	19-034,798
401	23040-L32623	*NINL*	20p11.21	TGGCCTGGGTTT-GCTGCTCCGGCA	20-025,441
247	06240-L05746	*DUSP15*	20q11.21	GATCACACACAT-CATCTCTATCCA	20-029,919
441	05916-L05361	*HSPA13*	21q11.2	ATTCAGCAAGTA-TTGAAAGAAGGC	21-014,668
254	05911-L05356	*SAMSN1*	21q11.2	CCCACAAATGGA-AGTGGAGAACAA	21-014,811
261 «	05742-L05180	*HDHD5* »	22q11.1	CTCTGAAAGCCT-TCCGAAGGCTGG	22-016,011
450	05741-L05219	*ADA2* »	22q11.1	GACGCTCAAAAT-CGCTGAGATGAA	22-016,070
409	05743-L05181	*UBQLN2*	Xp11.1	AGACACTCGAAA-TTGCCAGGAATC	X-056,608
268	23031-L32482	*ZC4H2*	Xq11.1	AGAATGACCTAA-ACAAGCTGCTAG	X-064,057

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.
« 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: NIPSNAP2 (GBAS); SPIDR (KIAA0146); HDHD5 (CECR5); ADA2 (CECR1).

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.

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

Related SALSA MLPA probemixes

P036 Subtelomeres Mix 1	Contains one probe for each of the 41 subtelomeric regions and 5 probes near the centromeric regions of the five acrocentric chromosomes.
P070 Subtelomeres Mix 2B	Contains one probe for each of the 41 subtelomeric regions and 5 probes near the centromeric regions of the five acrocentric chromosomes.
P095 Aneuploidy	Contains probes for chromosomes 13, 18, 21, X and Y.
P182 Centromere mix 2	Contains probes that detect the same regions but different sequences compared to the probes of P181 Centromere mix 1.

References

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Selected publications using SALSA MLPA Probemix P181 Centromere mix 1

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- Groeneveld-Krentz S et al. (2019). Aneuploidy in children with relapsed B-cell precursor acute lymphoblastic leukaemia: clinical importance of detecting a hypodiploid origin of relapse. *Br J Haematol*, 185(2), 266-283.
- Malvestiti F et al (2014) De novo small supernumerary marker chromosomes detected on 143,000 consecutive prenatal diagnoses: chromosomal distribution, frequencies, and characterization combining molecular cytogenetics approaches. *Prenat Diagn.* 34:460-8.
- 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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- Wu T et al. (2019). Evaluation of two aneuploidy screening tests for chorionic villus samples: Multiplex ligation-dependent probe amplification and fluorescence in situ hybridization. *Mol Cell Probes*, 46, 101422.
- Zhu Y et al. (2017). Cell cycle and histone modification genes were decreased in placenta tissue from unexplained early miscarriage. *Gene*, 636, 17-22.
- Zimowski JG et al. (2016) First-trimester spontaneous pregnancy loss – molecular analysis using multiplex ligation-dependent probe amplification. *Clin Genet*. 89: 620-624.

P181 product history	
Version	*Modification*
C1	Four probes changed in length but not in sequence detected and 19 probes were replaced.
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.

Implemented changes in the product description

Version C1-01 – 10 August 2021 (04P)
- Product description rewritten and adapted to a new template.
- Product descriptions for P181 Centromere mix 1 and P182 Centromere mix 2 are separated.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- The following gene names have been adjusted: *KIAA0146*, *CECR5*, *GBAS*, and *CECR1* (see Tables 1 and 2).
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Sections “Related SALSA MLPA Probemixes” and “Selected publications” were updated.

Version B2-02 – 26 March 2021 (01P)
- Chromosomal bands for *EPHA3* in Table 1a and 1b corrected.

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).

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