

Product Description

SALSA® MLPA® Probemix P182-C1 Centromere mix 2

To be used with the MLPA General Protocol.

Version C1

As compared to version B2, six probes have been replaced and one probe has changed in length, not in the sequence detected. For complete product history see page 8.

Catalogue numbers:

- P182-025R: SALSA MLPA Probemix P182 Centromere mix 2, 25 reactions.
- P182-050R: SALSA MLPA Probemix P182 Centromere mix 2, 50 reactions.
- **P182-100R:** SALSA MLPA Probemix P182 Centromere mix 2, 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 P182 Centromere mix 2 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 P182-C1 Centromere mix 2 contains 46 MLPA probes with amplification products between 130 and 453 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 P181 Centromere mix 1 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 118 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)	
105-118	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 of the same sex 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 (\geq 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 different 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 FR of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:



Copy number status		
Autosomal sequences and X chromosome sequences in females	X chromosome sequences in males	Final ratio (FR)
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.

- <u>Arranging probes</u> 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.
- <u>False positive results</u>: 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.
- <u>Normal copy number variation</u> in healthy individuals is described in the database of genomic variants: <u>http://dgv.tcag.ca/dgv/app/home</u>. 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.
- <u>False results can be obtained if one or more peaks are off-scale</u>. 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 P182 Centromere Mix 2 are confirmed with P181 Centromere Mix 1. 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 P182-C1 Centromere Mix 2

Length (nt)	SALSA MLPA probe	Gene detected	Chromosomal position (hg18)	Location (hg18) in kb
64-118	Control fragments – see ta	ble in probemix content	section for more information	ì
130 *	23096-L32613	NOTCH2	1p12	01-120,331
136	05747-L05185	EIF2AK3	2p11.2	02-088,674
142	05917-L05362	EPHA3	3p11.1	03-089,539
147 «	15147-L16921	ZAR1	4p12	04-048,191
154	05751-L05189	HCN1	5p12	05-045,498
160 *	22642-L31854	RAB23	6p12.1	06-057,169
166	05755-L06276	SUMF2	7p11.2	07-056,110
172	06244-L06278	HOOK3	8p11.21	08-042,982
178 *	23097-L32614	EXOSC3	9p13.2	09-037,772
184	05760-L06666	ZNF25	10p11.21	10-038,286
190	05918-L05363	PTPRJ	11p11.2	11-048,106
196 *	23098-L32742	PKP2	12p11.21	12-032,913
202	05763-L05201	MPHOSPH8	13q12.11	13-019,141
208	05765-L05203	PARP2	14q11.2	14-019,895
214 «	15148-L11839	MAGEL2	15q11.2	15-021,440
220 *	23100-L32617	AHSP	16p11.2	16-031,447
226	15144-L16919	USP22	17p11.2	17-020,865
232	05772-L05210	RNMT	18p11.21	18-013,732
240	05774-L05212	GMIP	19p13.11	19-019,602
247	05775-L05720	ZNF337	20p11.21	20-025,615
255	05919-L05364	HSPA13	21q11.2	21-014,666
263	05777-L10758	ADA2 (CECR1)	22q11.1	22-016,068
269	05779-L28310	MAGEH1	Xp11.21	X -055,496
276	05746-L05184	HJV (HFE2)	1q21.1	01-144,128
283	05748-L05186	ZNF2	2q11.1	02-095,210
289	06238-L04687	PROS1	3q11.2	03-095,095
298	05750-L05188	USP46	4q12	04-053,189
305	05752-L05190	ISL1	5q11.2	05-050,716
311	06246-L05752	KHDRBS2	6q11.1	06-062,816
319	06805-L06400	ASL	7q11.21	07-065,192
328 ¥	21537-L25660	PRKDC	8q11.21	08-049,029
337	05759-L06281	TJP2	9q21.11	09-071,059
346	06218-L28309	RET	10q11.21	10-042,934
353	05761-L05721	APLNR	11q12.1	11-056,761
360	05762-L05200	KIF21A	12q12	12-037,975
369	05770-L05208	VPS35	16q11.2	16-045,260
379	05921-L05366	WSB1	17q11.1	17-022,663
387	05773-L05211	MIB1	18q11.2	18-017,678
393 «	15145-L17579	CCNE1	19q12	19-035,006
400 «	05781-L01485	REM1	20q11.21	20-029,528
409	05780-L05218	ARHGEF9	Xq11.1	X -062,792
418	05764-L05202	ZMYM2	13q12.11	13-019,506
427	06493-L17676	APEX1	14q11.2	14-019,995
432 *	23103-L32735	MKRN3	15q11.2	15-021,361
441	05776-L05723	SAMSN1	21q11.2	21-014,815
453	06220-L04860	SLC25A18	22q11.21	22-016,423

* 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.

Length	SALSA MLPA	Cono	Chromosomal	<u>Partial</u> sequence (24 nt	Location
(nt)	probe	Gene	position (hg18)	adjacent to ligation site)	(hg18) in kb
130	23096-L32613	NOTCH2	1p12	CAGTCATCAATA-TTCCTCTCACAG	01-120,331
276	05746-L05184	HJV »	1q21.1	GCTGAGGTGGAT-AATCTTCCTGTA	01-144,128
136	05747-L05185	EIF2AK3	2p11.2	TAGTGACGAAAT-GGAACAAGAGGA	02-088,674
283	05748-L05186	ZNF2	2q11.1	CTGATGTGATTT-TCCAATTGAAGA	02-095,210
142	05917-L05362	EPHA3	3p11.1	CCAAGTGGTCAT-GATCGCCATTTC	03-089,539
289 #	06238-L04687	PROS1	3q11.2	ATAGATTCTGCG-TACAGTATCACG	03-095,095
147 «	15147-L16921	ZAR1	4p12	ACTTCGCCACGT-GGACCCTAAACG	04-048,191
298	05750-L05188	USP46	4q12	CAGCAAAAGAAG-AAGGAAAACTTG	04-053,189
154	05751-L05189	HCN1	5p12	GTCTTCAGTTCT-TAGTACCACTAC	05-045,498
305	05752-L05190	ISL1	5q11.2	GGCAATCAGATT-CACGATCAGTAT	05-050,716
160	22642-L31854	RAB23	6p12.1	CCTATCTGTGGT-AGAGAACACGAG	06-057,169
311	06246-L05752	KHDRBS2	6g11.1	CAAGAGGAAACT-CCTTGAAGAGGC	06-062,816
166	05755-L06276	SUMF2	7p11.2	GCATCCGAGAGA-GACTGGAGCACC	07-056,110
319	06805-L06400	ASL	7g11.21	TTTGAAGTGTCA-GACACTATGAGT	07-065,192
172	06244-L06278	НООКЗ	8p11.21	TAATGAACTACA-GAAGAAGAGAGC	08-042.982
328	21537-L25660	PRKDC	8a11.21	GGTGAAGTTCAT-CCTAGTGAGATG	08-049.029
178	23097-L32614	EXOSC3	9p13.2	ACTITCTAATTA-AGCCCAGAGTCA	09-037.772
337	05759-L06281	TJP2	9a21.11	ATGCACCATGGA-GACGTGGTGGGA	09-071.059
184	05760-L06666	ZNF25	10p11.21	ATGTTATTGTGG-AATTCACCAAGG	10-038.286
346	06218-L28309	RET	10a11.21	CCTGCTGTCAGA-GTTCAACGTCCT	10-042.934
190	05918-L05363	PTPRJ	11p11.2	GGGGAGACAGAT-TCTTCCAATCTC	11-048.106
353	05761-L05721	APLNR	11a12.1	GGGGTAAGGCAA-GAGAGGGTGGAG	11-056.761
196	23098-L32742	PKP2	12p11.21	ATCTCCATGTCT-GCATTCCTAGAC	12-032.913
360	05762-1 05200	KIF21A	12a12		12-037.975
202	05763-L05201	MPHOSPH8	13a12.11	CTAGAACCAGTT-TTTCCAATCGCA	13-019.141
418	05764-L05202	ZMYM2	13a12.11	ACTTGTTCAGAT-GACTATAAGAAG	13-019.506
208	05765-L05203	PARP2	14a11.2	CTCTCGCCTAAA-GAATACAGGACT	14-019.895
427	06493-L17676	APEX1	14a11.2	AGACCTCAATGT-GGCACATGAAGA	14-019.995
432	23103-L32735	MKRN3	15a11.2	AGGGCTTACACT-GGATACGCTTTT	15-021.361
214 «	15148-L11839	MAGEL2	15a11.2	AGCAAGATGCTT-GTCCTGAGGTTT	15-021.440
220	23100-1 32617	AHSP	16p11.2	GCACCCTCAAGA-GTGTGGGTGAGA	16-031.447
369	05770-L05208	VPS35	16g11.2		16-045.260
226	15144-L16919	USP22	17p11.2		17-020.865
379	05921-L05366	WSB1	17a11.1	TGTCAATCCGAA-GAGTGATGCCCA	17-022.663
232	05772-1 05210	RNMT	18p11.21	ATGCTGAGAAAT-GCGTGTGAGAGA	18-013.732
387	05773-1 05211	MIB1	18g11.2		18-017.678
240	05774-1 05212	GMIP	19n13 11		19-019 602
393 «	15145-117579	CCNF1	19g12		19-035.006
247	05775-1 05720	ZNE337	20n11 21	TTGGGGATGTCA-CTGTGGATTTCA	20-025.615
400 «	05781-1 01485	RFM1	20p11.21		20-029 528
255	05919-105364	HSPA13	21g11 2		21-014 666
441	05776-1 05723	SAMSN1	21q11.2		21-014,000
262	05777-1 10758		21911.2 22a11.1		27-014,013
152	06220-1 0/960	SI C25A18	22411.1 22a11.21		22.016.000
260	05770-1 28210	MAGEU1	22411.21 Yn11.21		¥-055 /06
400	05790-105210		Υα11_1		A-033,490 V_062 702
409	00/00-200218	AKIGELA		ACATCIGITUTI-IGCCAAGAAGCI	A-002,/92

Table 2. P182-C1 probes arranged according to chromosomal location

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: HJV (HFE2); 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.
P181 Centromere mix 1	Contains probes that detect the same regions but different sequences
	compared to the probes of P182 Centromere mix 2.

References

- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol*. 147:60-8.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligationdependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P182 Centromere mix 2

- Chen CP et al (2010) Prenatal diagnosis and molecular cytogenetic characterization of a small supernumerary marker chromosome derived from chromosome 8. *Taiwan J Obstet Gynecol.* 49:500-5.
- Leone PE et al. (2020). De novo duplication of chromosome 9p in a female infant: Phenotype and genotype correlation. *J Pediatr Genet*, 9(01), 069-075.
- 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.
- 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.
- Schouten J et al. (2019). Multiplex ligation-dependent probe amplification (mlpa) for prenatal diagnosis of common aneuploidies. In *Prenat Diagn* (pp. 161-170). Humana Press, New York, NY.
- Vega-Garcia N et al. (2020). Helpful criteria when implementing ngs panels in childhood lymphoblastic leukemia. *J Pers Med*, 10(4), 244.



P182 product history		
Version	Modification	
C1	Six probes have been replaced and one probe has changed in length, not in the sequence detected.	
B2	Three probes changed in length and two denaturation control fragments (88 and 96 nt, QDX2) have been replaced.	
B1	Four probes have been replaced and four extra control fragments have been added.	
A1	First release.	

Implemented changes in the product description

Version C1-01 - 19 April 2022 (04P)

- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).

- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

- Section "Selected publications" was updated.

Version B2-02 - 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.

- The following gene names have been adjusted: CECR1 and HFE2 (see Tables 1 and 2).

- Warnings 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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