Product Description
SALSA® MLPA® Probemix P147-C1 1p36

To be used with the MLPA General Protocol.

Version C1
As compared to version B2, seven target probes were replaced, nine reference probes were replaced and one reference probe was added. Two target probes were changed in length but not in the sequence detected. For complete product history see page 7.

Catalogue numbers:
- **P147-025R:** SALSA MLPA Probemix P147 1p36, 25 reactions.
- **P147-050R:** SALSA MLPA Probemix P147 1p36, 50 reactions.
- **P147-100R:** SALSA MLPA Probemix P147 1p36, 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 P147 1p36 is a **research use only (RUO)** assay for the detection of deletions or duplications in the 1p36 subtelomeric region, which are associated with the 1p36 deletion syndrome, also referred to as "monosomy 1p36". The P147 1p36 can be used to confirm and further characterise abnormalities detected by P036 Subtelomeres Mix 1 and/or P070 Subtelomeres Mix 2B.

1p36 deletion syndrome is a chromosome disorder where the end of the short arm of one of the two chromosomes is lost. The breakpoints for this cytogenetic syndrome are variable and range from bands 1p36.13 to 1p36.33 (Slavotinek et al. 1999). It is considered to be one of the most common chromosome terminal deletion syndromes and is a frequent cause of intellectual disability, although there is phenotypic variability based on the location and the extent of the deletions (Jordan et al. 2015). The incidence has been estimated to be 1 in 5,000 to 1 in 10,000 live born children (Gajecka et al. 2007). To date, more affected females than males have been reported.

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/
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE

Probemix content
The SALSA MLPA Probemix P147-C1 1p36 contains 48 MLPA probes with amplification products between 124 and 500 nucleotides (nt). This includes 37 probes for the 1p36 subtelomeric region. In addition, 11 reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

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, 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
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 different unrelated individuals who are from families without a history of 1p36 deletion syndrome. 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 reference probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.
- Interpretation of abnormal copy number findings in subtelomeric regions is complicated. Subtelomeric copy number changes can also occur in unaffected individuals and the effect of a deletion or duplication will depend on the genes involved. A considerable number of abnormalities detected by a single probe may not be the cause of any phenotypic effect but can be due to a rare polymorphism or a copy number change which is also present in one of the parents. For some chromosome arms, even large subtelomeric deletions or duplications (>1 Mb) can be inherited without a clear phenotypic effect. For all abnormalities detected, we strongly recommend testing parents to determine whether the copy number aberration in the patient is de novo.

P147 specific note:

Please note that deletions or duplications of part of 1p36 can be present in healthy persons! In case of positive results, it is therefore strongly recommended to also test the parents. As an example, a duplication of approximately 1 Mb was detected in a DNA sample from a healthy individual with the P036 and P070 Subtelomeres probemixes. This duplication was further characterized with this P147 probemix. The duplication included all probes from *TNFRSF4* up to *GABRD* (Kathleen Claes, Ghent, personal communication).

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.

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.

Database of Genomic Variants

We strongly encourage users to deposit positive results in the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P147-C1 1p36

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
64-105	Control fragments – see table in probemix content section for more information			
124 *	Reference probe 15370-L13762	7q11		
130 «	**TNFRSF4 probe** 02269-L01761		**1p36.33**	01-001,137
136 *	**CHD5 probe** 09115-L09175		**1p36.31**	01-006,088
142 *	**CCNL2 probe** 23079-L32576		**1p36.33**	01-001,323
148 «	**GABRD probe** 13354-L14784		**1p36.33**	01-001,949
154 *	Reference probe 13560-L15872	19p13		
160 «	**GABRD probe** 04690-L04068		**1p36.33**	01-001,946
166	**TNFRSF1B probe** 00553-L00122		**1p36.22**	01-012,171
178	**GNB1 probe** 02890-L02511		**1p36.33**	01-001,747
184	**CASP9 probe** 02880-L02347		**1p36.21**	01-015,704
191 *	Reference probe 06057-L05512	4p16		
196 *	**EPHA8 probe** 23080-L32577		**1p36.12**	01-022,794
202	**SCNN1D probe** 04692-L04070		**1p36.33**	01-001,207
208 *	**RER1 probe** 23081-L32578		**1p36.32**	01-002,318
214	**AJAP1 probe** 04704-L04082		**1p36.32**	01-004,672
220 *	Reference probe 16368-L18761	12q13		
229 «	**AGRN probe** 04687-L04065		**1p36.33**	01-000,948
238	**ICMT probe** 04698-L04076		**1p36.31**	01-006,208
247 «	**PANK4 probe** 01122-L00680		**1p36.32**	01-002,443
255 * «	**TNFRSF18 probe** 23082-L32579		**1p36.33**	01-001,130
263	**GNB1 probe** 13358-L15767		**1p36.33**	01-001,766
270 *	Reference probe 17398-L32045	3p21		
276	**MTOR probe** 04679-L15769		**1p36.22**	01-011,091
283	**NPHP4 probe** 04700-L04078		**1p36.31**	01-005,969
292 *	**ACTRT2 probe** 23083-L32580		**1p36.32**	01-002,929
304 ¥	**KIF1B probe** 23090-L04451		**1p36.22**	01-010,358
313 *	Reference probe 06580-L24038	2q24		
319	**DFFB probe** 04696-L04074		**1p36.32**	01-003,790
327 «	**TP73 probe** 01682-L01262		**1p36.32**	01-003,558
337	**PLOD1 probe** 04685-L04063		**1p36.22**	01-011,943
346	**TNFRSF14 probe** 04693-L04071		**1p36.32**	01-002,480
355 «	**PRDM2 probe** 04702-L04080		**1p36.21**	01-013,904
364	**CAMTA1 probe** 04695-L04073		**1p36.23**	01-007,728
373 *	Reference probe 20867-L28885	21q21		
382 «	**PRDM2 probe** 03423-L04457		**1p36.21**	01-013,899
391	**SLC45A1 probe** 04697-L04075		**1p36.23**	01-008,327
399 «	**TNFRSF4 probe** 09198-L15771		**1p36.33**	01-001,138
410	**TNFRSF9 probe** 02185-L15770		**1p36.23**	01-007,923
418 *	Reference probe 09793-L25209	15q15		
427	**MTHFR probe** 04684-L04062		**1p36.22**	01-011,773
436 «	**SKI probe** 02891-L02359		**1p36.33**	01-002,227
445 *	Reference probe 16286-L18578	13q14		
454	**PARK7 probe** 02189-L02365		**1p36.23**	01-007,968
463	**KIF1B probe** 04680-L04059		**1p36.22**	01-010,215
469 *	Reference probe 20128-L27639	18p11		
481 ¥	**PRDM16 probe** 04703-L25622		**1p36.32**	01-003,151
490 * «	**ISG15 probe** 23084-L32581		**1p36.33**	01-000,939
500	Reference probe 17001-L22947	20q11		

* New in version C1.
¥ Changed in version C1. Minor alteration, no change in sequence detected.

Table 2. P147-C1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to p-telomere (hg18)	Distance to next probe
490 «	23084-L32581	*ISG15*	CAAGGGCCGCAG-CAGCACCTACGA	939 kb	8 kb
229 «	04687-L04065	*AGRN*	CCCCTCATCTGT-GACAACCAGGTG	947 kb	182 kb
255 «	23082-L32579	*TNFRSF18*	CACAGTCGATAC-ACTGGAAGCCAA	1.129 kb	8 kb
130 «	02269-L01761	*TNFRSF4*, exon 5 NM_003327.4	GCCGGCCAGCAA-TAGCTCGGACGC	1.137 kb	1 kb
399 «	09198-L15771	*TNFRSF4*, exon 3 NM_003327.4	AACTCCAGGCTT-GTAGCTGTCCAG	1.138 kb	69 kb
202	04692-L04070	*SCNN1D*	AGTGACGAAGCT-GTGATTCACACA	1.207 kb	116 kb
142	23079-L32576	*CCNL2*	CAAGACGCATAC-GGGACGTCATCA	1.323 kb	423 kb
178	02890-L02511	*GNB1*, exon 3 NM_002074.5	CTAAGATCGGAA-GATGAGTGAGCT	1.746 kb	19 kb
263	13358-L15767	*GNB1*, intron 1 NM_002074.5	AGTGGTGCACTT-ATGTGTTTCCCA	1.765 kb	181 kb
160 «	04690-L04068	*GABRD*, exon 2 NM_000815.5	CGGCGACTACGT-GGGCTCCAACCT	1.946 kb	3 kb
148 «	13354-L14784	*GABRD*, exon 6 NM_000815.5	TCATCGGAGGAC-ATCGTCTACTAC	1.949 kb	278 kb
436 «	02891-L02359	*SKI*	AACGAGAAGAAG-ATGAAAGAGGCC	2.227 kb	91 kb
208	23081-L32578	*RER1*	TACATGATTCGA-GTTTACCTGCTG	2.318 kb	125 kb
247 «	01122-L00680	*PANK4*	CTATTCAACGGT-ACAGCACAAAGT	2.443 kb	36 kb
346	04693-L04071	*TNFRSF14*	CAATACCCTCAT-TCACGGGGAGGA	2.479 kb	450 kb
292	23083-L32580	*ACTRT2*	CGGAGGTCCCAA-ACTCCTTGAAGT	2.929 kb	221 kb
481	04703-L25622	*PRDM16*	ACGGACGTGGAA-GTGTCGCCCCAG	3.150 kb	408 kb
327 «	01682-L01262	*TP73*	GAGACCCGGGTG-TCAGGAAAGATG	3.558 kb	232 kb
319	04696-L04074	*DFFB*	TGCACATTGTCT-GCCATAAGAAAA	3.790 kb	0,9 **M**b
214	04704-L04082	*AJAP1*	TGATAGCCATGT-TTCAGCTCGCCG	4.671 kb	1,3 **M**b
283	04700-L04078	*NPHP4*	GGATGAACGACT-GGCACAGGATCT	5.968 kb	119 kb
136	09115-L09175	*CHD5*	TGCAGCACTGAT-GTCTCTTTACCG	6.087 kb	120 kb
238	04698-L04076	*ICMT*	CAGCTATGCCCT-GACAGTGTGGCG	6.207 kb	1,5 **M**b
364	04695-L04073	*CAMTA1*	AATGAGCTGGCT-GGCCAGTTATCT	7.727 kb	195 kb
410	02185-L15770	*TNFRSF9*	GGTCCTCAACTT-TGAGAGGACAAG	7.922 kb	45 kb
454	02189-L02365	*PARK7*	AGAGCAGCGAAC-TGCGACGATCAC	7.967 kb	359 kb
391	04697-L04075	*SLC45A1*	CAACGGGGTGAT-GTACTTCTCCAG	8.326 kb	1,9 **M**b
463	04680-L04059	*KIF1B*, exon 2 NM_001365951.3	CTCAGTGAAGGT-GGCTGTCCGGGT	10.214 kb	143 kb
304	23090-L04451	*KIF1B*, exon 48 (46) NM_001365951.3	CGTGGGGTCCTT-TTGCAGGCCCTC	10.357 kb	733 kb
276	04679-L15769	*MTOR*	TCCAACGCAAGT-TGAGCTGCTCAT	11.090 kb	683 kb
427	04684-L04062	*MTHFR*	TGACTTCCCACT-GGACAACTGCCT	11.773 kb	170 kb
337	04685-L04063	*PLOD1*	GCATGGCAGCGA-GTACCAGTCTGT	11.943 kb	228 kb
166	00553-L00122	*TNFRSF1B*	GGCTCAGAGAAT-ACTATGACCAGA	12.171 kb	1,7 **M**b
382 «	03423-L04457	*PRDM2*, upstream NM_012231.5	GCCATTGGGCGA-CGGCGCAGGGTC	13.899 kb	5 kb
355 «	04702-L04080	*PRDM2*, exon 1 NM_012231.5	TTGACCTTCCCT-CCACTCTTACAG	13.904 kb	1,8 **M**b
184	02880-L02347	*CASP9*	GGTCGAGAAGAT-TGTGAACATCTT	15.703 kb	7,1 **M**b
196	23080-L32577	*EPHA8*	GCCTGACGCTCA-TCACGGGCCTGG	22.794 kb	

[a] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« 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.

The exon numbering used in previous versions of this product description can be found in between brackets.

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.

Related products
For related products, see the product page on our website.

References

- Gajecka M et al. (2007). Monosomy 1p36 deletion syndrome. *Am J Med Genet Part C*. 145C:346-356.
- Jordan VK et al. (2015). 1p36 deletion syndrome: an update. *Appl Clin Genet*. 8:189-200.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent 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.
- Slavotinek A et al. (1999). Monosomy 1p36. *J Med Genet*. 36:657-663.
- 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 P147 1p36

- D'Angelo CS et al. (2006) Prader-Willi-like phenotype: investigation of 1p36 deletion in 41 patients with delayed psychomotor development, hypotonia, obesity and/or hyperphagia, learning disabilities and behavioral problems. *Eur J Med Genet*. 49(6):451-60.
- D'Angelo CS et al. (2009). Extending the phenotype of monosomy 1p36 syndrome and mapping of a critical region for obesity and hyperphagia. *Am J Med Genet Part A*. 152A:102-110.
- Hirschfeldova K et al. (2011). Cryptic chromosomal rearrangements in children with idiopathic mental retardation in the Czech population. *Genetic Testing and Molecular Biomarkers*. 15:607-611.
- Xu F et al. (2014) The first patient with a pure 1p36 microtriplication associated with severe clinical phenotypes. *Mol Cytogenet*. 7(1):64.

P147 product history	
Version	*Modification*
C1	Seven target probes and nine reference probes have been replaced and one reference probe has been added. Two target probes changed in length, but not in sequence detected.
B2	Four reference probes were replaced and the control fragments were adjusted (QDX2).
B1	Four probes on 1p36 were removed and five new probes were included. In addition, four extra control fragments at 88, 96, 100 and 105 nt were added.
A1	First release.

Implemented changes in the product description
Version C1-02– 28 April 2025 (04P) - Gene structure and transcript variants section: link to MANE website added. - NM sequence of the KIF1B probes updated. - Exon numbering of the 304 nt probe changed. - Removed Related SALSA MLPA products section. - Small layout changes. Version C1-01– 09 August 2021 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).

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