

Product Description

SALSA® MLPA® Probemix P389-B1 MLL2

To be used with the MLPA General Protocol.

Version B1

For complete product history see page 7.

Catalogue numbers

- **P389-025R:** SALSA® MLPA® Probemix P389 MLL2, 25 reactions
- **P389-050R:** SALSA® MLPA® Probemix P389 MLL2, 50 reactions
- **P389-100R:** SALSA® MLPA® Probemix P389 MLL2, 100 reactions

SALSA® MLPA® Probemix P389 MLL2 (hereafter: P389 MLL2) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P389-025R	P389-050R	P389-100R	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		
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A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis

Information regarding 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

SALSA® MLPA® Probemix P389 MLL2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MLL2* gene, which is associated with Kabuki Syndrome (KS).

KS is a rare syndrome characterised by intellectual disability and multiple congenital abnormalities, in particular a distinct dysmorphic facial appearance. KS can be caused by mutations in the *MLL2* gene (also known as *KMT2D*), encoding an H3K4 histone methyl transferase which acts as an epigenetic transcriptional

activator during growth and development. The vast majority of reported cases have been sporadic, but parent-to-child transmission suggests that KS is an autosomal dominant disorder.

More information is available at <https://www.ncbi.nlm.nih.gov/books/NBK62111/>.

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:

Entrez Gene shows transcript variants of each gene: <https://www.ncbi.nlm.nih.gov/gene>

For NM_ mRNA reference sequences: <https://www.ncbi.nlm.nih.gov/nucleotide>

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

Tark – Transcript Archive: <https://tark.ensembl.org>

Exon numbering

The *MLL2* exon numbering used in this P389-B1 *MLL2* product description is the exon numbering derived from MANE project (release version 1.5) based on MANE Select transcript NM_003482.4. The *MLL2* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version B1-03 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select NM_ sequence for this gene. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content

P389-B1 *MLL2* contains 35 MLPA probes with amplification products between 136 and 463 nucleotides (nt). This includes 26 probes for the *MLL2* gene. In addition, nine 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 MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation

Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families without a history of Kabuki 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 deviations to the indicated copy number alteration (CNA) findings might occur.

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 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/gain	$1.30 < FR < 1.65$
Heterozygous triplication/homozygous duplication/gain	$1.75 < FR < 2.15$
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 Coffalyser.Net. (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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: <https://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** 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *MLL2* gene are small (point) mutations, none of which will be detected by using P389 MLL2.
- 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 in sequence data 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.

MLL2 (*KMT2D*) mutation database

<https://databases.lovd.nl/shared/genes/KMT2D>. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on <https://varnomen.hgvs.org>.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *MLL2* exons 34 and 36 but not exon 35) to MRC Holland: info@mrcholland.com.

Table 1. P389-B1 MLL2

Length (nt)	MLPA probe	Chromosomal position (hg18) ^a	
		Reference	<i>MLL2</i>
64-105	Control fragments – see table in probemix content section for more information		
136	Reference probe 05747-L05185	2p	
142	MLL2 probe 16358-L18751		Exon 5
148	MLL2 probe 16359-L18752		Exon 55
154 Δ	MLL2 probe 16360-L18753		Exon 11
160	MLL2 probe 16361-L18754		Exon 35
172	MLL2 probe 16362-L18755		Exon 20
178 Ж	MLL2 probe 16363-SP0358-L18756		Exon 55
184	MLL2 probe 16364-L18757		Exon 8
190	MLL2 probe 16365-L18758		Exon 48
196	Reference probe 10723-L11305	6p	
207	MLL2 probe 16366-L18759		Exon 40
213	MLL2 probe 16367-L18760		Exon 31
220	MLL2 probe 16368-L18761		Exon 15
228 Ж	MLL2 probe 16369-SP0359-L18762		Exon 52
234	Reference probe 11156-L16377	5q	
247	MLL2 probe 16370-L18763		Exon 27
255	MLL2 probe 16371-L18764		Exon 44
265	MLL2 probe 16372-L18765		Exon 34
272	MLL2 probe 16373-L18766		Exon 2
283	Reference probe 04404-L02610	14q	
301	MLL2 probe 16375-L18768		Exon 12
310	Reference probe 09245-L09435	7q	
317	MLL2 probe 16377-L18770		Exon 23
328	Reference probe 10857-L11527	9p	
337 Ж	MLL2 probe 16378-SP0360-L18771		Exon 46
355	MLL2 probe 16380-L18773		Exon 55
364	MLL2 probe 16381-L18774		Exon 36
375	Reference probe 13585-L15042	1q	
400	MLL2 probe 16384-L18777		Exon 16
415	Reference probe 09070-L09239	19p	
424	MLL2 probe 16386-L18779		Exon 35
436	MLL2 probe 16387-L18780		Exon 50
447 Ж	MLL2 probe 16388-SP0362-L18781		Exon 32
454	MLL2 probe 16389-L18782		Exon 42
463	Reference probe 14308-L15978	15q	

^a See section Exon numbering on page 2 for more information.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. *MLL2* probes arranged according to chromosomal location

Length (nt)	MLPA probe	<i>MLL2</i> exon ^a	Ligation site ^b NM_003482.4	Partial sequence ^c (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	1220-1222 (Exon 2)		
272	16373-L18766	Exon 2 (1)	1250-1251	CTGGTGAGGATA-AAGATTCAGAAC	0.9 kb
142	16358-L18751	Exon 5 (4)	1638-1639	CTGGGCTCACCA-TTGGTGTGCTGC	1.2 kb
184	16364-L18757	Exon 8 (7)	2127-2128	TACTTTCTGCCT-AAAACCAACCCAT	0.9 kb
154 Δ	16360-L18753	Exon 11 (10)	2523-2524	GGCCCCCTAAA-CGAGGAGATGCC	2.2 kb
301	16375-L18768	Exon 12 (11)	4605-4606	GGATGGGATTGA-TGCTCCGGGTTT	2.2 kb
220	16368-L18761	Exon 15 (14)	5420-5421	GTTTCGAGTGCT-CTCAGTGCTATC	1.3 kb
400	16384-L18777	Exon 16 (15)	5581-5582	AGTACCACACA-TACTGCCTGGAC	1.8 kb
172	16362-L18755	Exon 20 (19)	6008-6009	CAGAACTGGCA-TGGCCTTGCTGC	1.0 kb
317	16377-L18770	Exon 23 (22)	6448-6449	GCCGTGGAGCAG-AGCTTAGCTGAA	1.1 kb
247	16370-L18763	Exon 27 (26)	6899-6900	ACCTGCAGCAGC-TCTTCAAGGATG	1.2 kb
213	16367-L18760	Exon 31 (30)	7413-7414	GCAAAAGGCCAA-AGATAACCGGGC	1.2 kb
447 Ж	16388-SP0362-L18781	Exon 32 (31)	8508-8509; 8538-8539	ACCCCGGCCTCT-30 nt spanning oligo-TACCCCTCGCTT	1.1 kb
265	16372-L18765	Exon 34 (33)	9450-9451	TCTCTCTAGGA-CAAGAGCAGCCT	1.0 kb
160	16361-L18754	Exon 35 (34)	10207-10208	CTGGATGACGAT-TTTGATGCCAC	1.1 kb
424	16386-L18779	Exon 35 (34)	11302-11303	AGTGGGCAGCAT-GGAGGGCAGGCA	2.4 kb
364	16381-L18774	Exon 36 (35)	11492-11493	TTGATCCATTG-CAAAGGCCAAGA	2.1 kb
207	16366-L18759	Exon 40 (39)	13154-13155	TGGGCCTTTTAA-ACCAGAGTCGAA	2.0 kb
454	16389-L18782	Exon 42 (41)	14940-14941	CGCCAATTTTAG-CCTCTTTGCCCC	1.3 kb
255	16371-L18764	Exon 44 (43)	15288-15289	TCAGACTGAGGA-TGTCAGGTAGGG	0.5 kb
337 Ж	16378-SP0360-L18771	Exon 46 (45)	15529-15530; 15559-15560	GGAAAGCTGCCT-30 nt spanning oligo-GAGGTGTCAGTC	1.0 kb
190	16365-L18758	Exon 48 (47)	15791-15792	CTGATTGGCTGA-AGCAGTTTGATG	2.9 kb
436	16387-L18780	Exon 50 (49)	17021-17022	GGAATCGCATCA-TTGAGCCTGTGG	2.3 kb
228 Ж	16369-SP0359-L18762	Exon 52 (51)	17482-17483; 17517-17518	GAAAAGCACACA-35 nt spanning oligo-CGAGGTGGCCAA	0.8 kb
355	16380-L18773	Exon 55 (54)	17743-17744	TCTTTTCAGCTA-ACCTATGACTAT	0.1 kb
148	16359-L18752	Exon 55 (54)	17821-17822	AATTGTCGAAA-TGGATGAAGTAA	0.9 kb
178 Ж	16363-SP0358-L18756	Exon 55 (54)	18703-18704; 18736-18737	TGTTGAGGCAA-33 nt spanning oligo-GTTGCTCTAGGA	
		<i>stop codon</i>	17831-17833 (Exon 55)		

^a See section Exon numbering on page 2 for more information.

^b Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.

^c Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

SNVs located in the target sequence of a probe can influence probe hybridisation 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

- 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.
- 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 P389 MLL2

- Banka S et al. (2013). MLL2 mosaic mutations and intragenic deletion–duplications in patients with Kabuki syndrome. *Clin Genet*, 83(5), 467-471.
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- Priolo M et al. (2012). Absence of deletion and duplication of MLL2 and KDM6A genes in a large cohort of patients with Kabuki syndrome. *Mol Genet Metab*, 107(3), 627-629.
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- So PL et al. (2021). Clinical and molecular characterization study of Chinese Kabuki syndrome in Hong Kong. *Am J Med Genet A*, 185(3), 675-686.
- Usluer, E et al. (2022). Investigation of genetic and phenotypic heterogeneity in 37 Turkish patients with Kabuki and Kabuki-like phenotype. *Am J Med Genet A*.

P389 product history	
Version	Modification
B1	One probe detecting <i>MLL2</i> exon 39 has been removed.
A1	First release.

Implemented changes in the product description
Version B1-03 – 03 July 2026 (05P) - Product description rewritten and adapted to a new template. - Exon numbering of the <i>MLL2</i> gene has been changed. Version B1-02 – 22 September 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the <i>MLL2</i> gene updated according to new version of the NM_ reference sequence. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). - Remark added for exon 10 probe in table 1 and 2, this probe may be more sensitive to certain experimental variations.

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