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.

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 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 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/

Exon numbering
The *MLL2* exon numbering used in this P389-B1 MLL2 product description is the exon numbering from the NG_027827.1 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the NG sequences. 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
The SALSA MLPA Probemix 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 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 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 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 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.

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 SALSA MLPA Probemix 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 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 http://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 33 and 35 but not exon 34) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P389-B1 MLL2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*MLL2*
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 4**
148	**MLL2 probe** 16359-L18752		**Exon 54**
154 Δ	**MLL2 probe** 16360-L18753		**Exon 10**
160	**MLL2 probe** 16361-L18754		**Exon 34**
172	**MLL2 probe** 16362-L18755		**Exon 19**
178 Ж	**MLL2 probe** 16363-SP0358-L18756		**Exon 54**
184	**MLL2 probe** 16364-L18757		**Exon 7**
190	**MLL2 probe** 16365-L18758		**Exon 47**
196	Reference probe 10723-L11305	6p	
207	**MLL2 probe** 16366-L18759		**Exon 39**
213	**MLL2 probe** 16367-L18760		**Exon 30**
220	**MLL2 probe** 16368-L18761		**Exon 14**
228 Ж	**MLL2 probe** 16369-SP0359-L18762		**Exon 51**
234	Reference probe 11156-L16377	5q	
247	**MLL2 probe** 16370-L18763		**Exon 26**
255	**MLL2 probe** 16371-L18764		**Exon 43**
265	**MLL2 probe** 16372-L18765		**Exon 33**
272	**MLL2 probe** 16373-L18766		**Exon 1**
283	Reference probe 04404-L02610	14q	
301	**MLL2 probe** 16375-L18768		**Exon 11**
310	Reference probe 09245-L09435	7q	
317	**MLL2 probe** 16377-L18770		**Exon 22**
328	Reference probe 10857-L11527	9p	
337 Ж	**MLL2 probe** 16378-SP0360-L18771		**Exon 45**
355	**MLL2 probe** 16380-L18773		**Exon 54**
364	**MLL2 probe** 16381-L18774		**Exon 35**
375	Reference probe 13585-L15042	1q	
400	**MLL2 probe** 16384-L18777		**Exon 15**
415	Reference probe 09070-L09239	19p	
424	**MLL2 probe** 16386-L18779		**Exon 34**
436	**MLL2 probe** 16387-L18780		**Exon 49**
447 Ж	**MLL2 probe** 16388-SP0362-L18781		**Exon 31**
454	**MLL2 probe** 16389-L18782		**Exon 41**
463	Reference probe 14308-L15978	15q	

[a] See section Exon numbering on page 1 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.

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. *MLL2* probes arranged according to chromosomal location

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

[a] See section Exon numbering on page 1 for more information.
[b] Only partial probe sequences are shown. 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 hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P445 KDM6A Contains probes for the *KDM6A* gene, involved in Kabuki syndrome type 2.

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 SALSA MLPA Probemix 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.
- Bögershausen N & Wollnik B. (2013). Unmasking kabuki syndrome. *Clin Genet*, 83(3), 201-211.
- Micale L et al. (2014). Molecular analysis, pathogenic mechanisms, and readthrough therapy on a large cohort of K abuki syndrome patients. *Hum Mutat*, 35(7), 841-850.
- Paděrová J et al. (2016). Molecular genetic analysis in 14 Czech Kabuki syndrome patients is confirming the utility of phenotypic scoring. *Clin Genet*, 90(3), 230-237.
- 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.
- Rieß A et al. (2012). Mirror-image asymmetry in monozygotic twins with kabuki syndrome. *Mol Syndromol*, 3(2), 94-97.
- 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 *MLL2* exon 39 has been removed.
A1	First release.

Implemented changes in the product description
Version B1-02 – 22 September 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *MLL2* 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. Version B1-01 – 09 January 2019 (01P) - Product description restructured 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	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200