

Product Description

SALSA® MLPA® Probemix P496-A1 KMT2A

To be used with the MLPA General Protocol.

Version A1

For complete product history see page 12.

Catalogue numbers:

- **P496-025R:** SALSA MLPA Probemix P496 KMT2A, 25 reactions.
- **P496-050R:** SALSA MLPA Probemix P496 KMT2A, 50 reactions.
- **P496-100R:** SALSA MLPA Probemix P496 KMT2A, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, SD096 Binning DNA 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 P496 KMT2A is a **research use only (RUO)** assay for the detection of deletions or duplications in the *KMT2A* gene. This probemix can also be used to detect the presence of the *ASXL1* c.1934dupG mutation.

The histone methyltransferase *KMT2A* methylates lysine 3 on histone 4 (H3K4), thereby shaping the epigenetic landscape in cells, modulating chromatin accessibility and transcription. A subset of leukemias is characterised by *KMT2A* alterations, which typically include amplifications, partial tandem duplications (PTDs) and translocations (Rao et al. 2015). *KMT2A* alterations are observed in 5-20% of Acute Myeloid Leukemia (AML) cases, and are associated with poor outcome (Choi et al. 2018; Issa et al. 2021; Yuen et al. 2023). *KMT2A* PTDs (also known as *MLL* PTDs) are observed in 6-7% of Myelodysplastic Syndrome (MDS) as well, and alongside *TP53* and *FLT3* mutations are considered as top genetic predictors of adverse outcomes according to IPSS-M (Molecular International Prognostic Scoring System for Myelodysplastic Syndromes) (Bernard E et al. 2022; Choi et al. 2018). Accurate detection of *KMT2A* PTDs using next-generation sequencing (NGS) or fluorescence *in situ* hybridisation (FISH) approaches remains difficult (McKerrell et al. 2016; Afrin et al. 2018; Dai et al. 2021; Tsai et al. 2022), whereas MLPA technique was shown to yield robust and reliable results with a short turnaround time (Balgobind et al. 2010; Kentaro et al. 2013) and allows more accurate detection of *KMT2A* PTDs when combined with DNA structural analysis approaches (Capo-Chichi et al. 2022).

ASXL1 is a gene that is frequently mutated in various haematological malignancies, including AML and MDS. The most common genetic alteration found in the *ASXL1* gene is the c.1934dupG mutation, which leads to a premature stop codon in the *ASXL1* transcript, resulting in a truncated *ASXL1* protein which is rapidly degraded. Importantly, loss-of-function *ASXL1* mutations were shown to promote myeloid transformation through loss of PRC2-mediated gene repression (Abdel-Wahab et al. 2012; Gelsi-Boyer et al. 2012). The *ASXL1* c.1934dupG mutation appears to be mostly restricted to the haematopoietic lineage, and is associated with poor prognosis in AML and MDS patients (Gelsi-Boyer et al. 2012).

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>

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

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

Locus Reference Genomic (LRG) database: <http://www.lrg-sequence.org/>

Exon numbering

The *KMT2A* exon numbering used in this P496-A1 *KMT2A* product description is the exon numbering from the MANE project (release version 1.0) based on the MANE Select transcript NM_001197104.2, which can be found in Tables 1 and 2. The *TP53* exon numbering is derived from the MANE project based on NM_000546.6. 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. Please note that exon numbering for the same gene might be different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content

The SALSA MLPA Probemix P496-A1 *KMT2A* contains 60 MLPA probes with amplification products between 64 and 505 nucleotides (nt). This includes 17 probes for the *KMT2A* gene and two probes upstream and downstream of *KMT2A* gene, namely for the *UBE4A* and *TMEM25* genes. This probemix also contains one probe specific for the *ASXL1* c.1934dupG mutation which will only generate a signal when the mutation is present. Furthermore, probes were added for copy number determination of other regions commonly affected by CNAs in AML and MDS. These include two probes targeting *CTNNA1* and *NPM1* (5q deletions), one flanking probe for 5p, two probes for *IKZF1*, *CUX1*, *KMT2E* and *EZH2* (chromosome 7 and 7q deletions); three probes for *ATM* (11q deletions), two flanking probes for the 11p arm, one flanking probe for 11q, three probes for *TP53* (17p deletions), two probes for *NF1* and one for *SUZ12* (17q deletions). In addition, 13 reference probes are included that detect autosomal chromosomal locations and target relatively copy number stable regions in various cancer types including hematological cancers. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com) and in Table 3.

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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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 type or 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.

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 healthy individuals without a history of cancer. 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

See the section Positive samples on the [P496 KMT2A product page](#) on our website.

SALSA Binning DNA SD096

The SD096 Binning DNA provided with this probemix can be used for binning of all probes including the mutation-specific probe (ASXL1 probe 18261-SP0848-L2626 for the c.1934dupG mutation). SD096 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 μ l SD096 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD096 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

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 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 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 subclonal cases.
- 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.

P496-specific note

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Limitations of the procedure

- In AML and MDS, genetic defects affecting the *KMT2A* gene typically comprise CNAs and translocations. However, point mutations in *KMT2A* may also occur, most of which will not be detected by using SALSA MLPA Probemix P496-A1 *KMT2A*.
- 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. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in tumours with more chaotic karyotypes.

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.

Mutation database

LOVD mutation database: <https://databases.lovd.nl/shared/genes/KMT2A>; COSMIC mutation database: <https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=KMT2A>. We strongly encourage users to deposit positive results in the LOVD and COSMIC Databases. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on <http://varnomen.hgvs.org/>.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *KMT2A* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P496-A1 KMT2A

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a			Location (hg18) in kb
		Reference	KMT2A, at 11q23.3	Target region / mutation	
64-105	Control fragments – see table in probemix content section for more information				
121	Reference probe 19616-L27455	4p13			04-042,278
126	Reference probe S0959-L26042	9q21			09-078,133
130 «	KMT2A probe 23318-L32964		Exon 1		11-117,812
136 ~	TMEM25 probe 23319-L32965			11q23.3	11-117,908
139	TP53 probe 08304-L26800			17p13.1	17-007,518
145	KMT2A probe 23320-L32966		Exon 7		11-117,858
148	Reference probe 17858-L23867	2p21			02-044,401
154	NF1 probe 12018-L32953			17q11.2	17-026,708
160 § ± Ж	ASXL1 probe 18261-SP0848-L26260			c.1934dupG	20-030,486
166	KMT2A probe 23321-L32967		Exon 31		11-117,888
172	Reference probe 21100-L29489	1q32			01-199,330
177 ~	TMEM25 probe 23322-L32968			11q23.3	11-117,910
182 ~	ETS1 probe 09496-L25025			11q24.3	11-127,836
186	KMT2A probe 19885-L29240		Exon 36		11-117,898
193 ~	UBE4A probe 23323-L32969			11q23.3	11-117,767
197	KMT2A probe 09499-L32954		Exon 3		11-117,848
203 ~	NIPBL probe 04827-L32994			5p13.2	05-036,997
208	Reference probe 13384-L25019	6q12			06-064,999
215	NUP98 probe 06675-L06253			11p15.4	11-003,669
228	KMT2A probe 23334-L33002		Exon 4		11-117,853
233	Reference probe 15154-L25342	18q21			18-056,191
240	CUX1 probe 19886-L26753			7q22.1	07-101,527
246	EZH2 probe 18267-L26041			7q36.1	07-148,161
251 ~	UBE4A probe 23325-L32971			11q23.3	11-117,769
256	TP53 probe 02376-L30912			17p13.1	17-007,519
264	IKZF1 probe 13873-L15917			7p12.2	07-050,412
269 «	CUX1 probe 09472-L23871			7q22.1	07-101,664
274	Reference probe 09792-L23872	15q15			15-042,654
280	KMT2A probe 19132-L25682		Exon 5		11-117,854
285	NPM1 probe 18521-L25083			5q35.1	05-170,752
290	ATM probe 08445-L23873			11q22.3	11-107,741
296	SLC6A5 probe 08936-L33053			11p15.1	11-020,586
303 Ø	KMT2A probe 23335-L32983		Intr. 1		11-117,824
308	EZH2 probe 18272-L23874			7q36.1	07-148,143
315	Reference probe 14763-L24999	1q23			01-159,404
321	KMT2A probe 23326-L32972		Exon 3		11-117,850
328	CTNNA1 probe 18523-L23814			5q31.2	05-138,188
337	KMT2A probe 23327-L32973		Exon 2		11-117,845
346	ATM probe 02663-L22101			11q22.3	11-107,649
353	KMT2A probe 23328-L32974		Exon 10		11-117,861
358 «	KMT2A probe 23329-L32998		Exon 1		11-117,813
364	NPM1 probe 18527-L23818			5q35.1	05-170,750
371	ATM probe 08420-L22087			11q22.3	11-107,630
378	Reference probe 08141-L24356	14q11			14-020,863
384	KMT2E probe 18528-L24355			7q22.2	07-104,505
391	KMT2A probe 23330-L32996		Exon 6		11-117,856
401	TP53 probe 17423-L32981			17p13.1	17-007,518
407	Reference probe 01237-L24913	10p14			10-012,019
412	NF1 probe 18544-L24995			17q11.2	17-026,612
418	KMT2E probe 18796-L24994			7q22.2	07-104,490

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a			Location (hg18) in kb
		Reference	<i>KMT2A</i> , at 11q23.3	Target region / mutation	
436	<i>KMT2A</i> probe 23331-L32977		Exon 13		11-117,866
443	<i>KMT2A</i> probe 19133-L32999		Exon 3		11-117,848
452	Reference probe 16286-L27983	13q14			13-050,429
459	<i>CTNNA1</i> probe 18533-L32980			5q31.2	05-138,117
466	<i>KMT2A</i> probe 23332-L32978		Exon 9		11-117,860
472	<i>IKZF1</i> probe 14061-L24990			7p12.2	07-050,423
484	<i>KMT2A</i> probe 23333-L32979		Exon 19		11-117,872
489	Reference probe 17939-L15290	3q25			03-157,716
498	<i>SUZ12</i> probe 18539-L23830			17q11.2	17-027,340
505	Reference probe 15203-L23403	3p12			03-081,775

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

§ Mutation-specific probe. This probe will only generate a signal when the *ASXL1* c.1934dupG mutation is present. It has been tested on artificial DNA **and on DNA from the KASUMI-1 cell line**.

‡ An unspecific peak might be detected at one nucleotide shorter length from the expected length of this *ASXL1* c.1934dupG mutation-specific probe at 160 nt. Please analyse the peak pattern carefully when making calls for this mutation-specific probe.

« 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.

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.

– Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

Table 2. *KMT2A* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / exon ^a	Location / ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
Chromosome 5q deletions; <i>NIPBL</i> (5p13.2), <i>CTNNA1</i> (5q31.2) and <i>NPM1</i> (5q35.1).					
Chromosome 5 CNAs are common in AML and MDS (Walter et al. 2009). Deletions affecting the 5q-arm are recurrent genetic aberrations observed in approximately 10-15% of AML and MDS cases. In MDS, isolated 5q deletions are associated with a good prognosis. In AML, 5q deletions often occur in conjunction with 7q deletions as part of a complex karyotype, associated with a poor outcome (Venugopal et al. 2021). Genes located on 5q with a described role in myeloid malignancies include <i>CTNNA1</i> (Mrozek et al. 2008; Huang et al. 2023) and <i>NPM1</i> (Mazzola et al. 2019; Garfinkle and Gruber 2019). <i>NPM1</i> is also affected by exon 12 mutations in AML patients (La Starza et al. 2010; Falini et al. 2005; Berger et al. 2006). This problemix contains two <i>NPM1</i> probes for copy number detection, but not for point mutations.					
203 –	04827-L32994	<i>NIPBL</i>	5p13.2	CAACAAACCACT-ATCTCACATAGC	101,7 Mb
459	18533-L32980	<i>CTNNA1</i> , ex 1	NM_001903.5; 85-86	CAGCTAGCCGCA-GGTAACCTCGTA	71,3 kb
328	18523-L23814	<i>CTNNA1</i> , ex 6	NM_001903.5; 938-939	ATGCACTCAATA-ACTTTGACGTAA	32,3 Mb
364	18527-L23818	<i>NPM1</i> , ex 2	NM_002520.7; 7 nt before exon 2	CTTTTTCTTCA-TTACAGGTTGT	2,8 kb
285	18521-L25083	<i>NPM1</i> , ex 5	NM_002520.7; 551-552	GTGGTAGCAAGG-TTCCACAGGTAG	-
Chromosome 7 or 7q deletions; <i>IKZF1</i> (7p12.2), <i>CUX1</i> (7q22.1), <i>KMT2E</i> (7q22.2) and <i>EZH2</i> (7q36.1).					
Loss of chromosome 7 or deletion of 7q is one of the most common alterations found in myeloid malignancies, occurring in 10-20% of cases. Complete loss of chromosome 7 in AML and MDS is associated with a poor prognosis, whereas 7q deletions are associated with intermediate risk (El-Menoufy et al. 2018). Genes located on chr. 7 which have been postulated to be relevant in the context of AML include the tumour suppressors <i>IKZF1</i> , <i>CUX1</i> , <i>KMT2E</i> (also known as <i>MLL5</i>) and <i>EZH2</i> (Heuser et al. 2009; de Rooij et al. 2015; Skoda and Schwaller 2019).					
264	13873-L15917	<i>IKZF1</i> , ex 4	NM_006060.6; 477-478	TACGAATGCTTG-ATGCCTCGGGAG	10,7 kb
472	14061-L24990	<i>IKZF1</i> , ex 6	NM_006060.6; 812-813	TTTTCTGCAGTT-GGTAACCTCAC	51,9 Mb
240	19886-L26753	<i>CUX1</i> , ex 5	NM_181552.4; 367-368	GAACCAGAACT-TAGGGAACTCT	136,7 kb
269 «	09472-L23871	<i>CUX1</i> , ex 22	NM_181552.4; 3596-3595, reverse	AGCTTCTCCACA-TTGTGGGGTCG	2,7 Mb

Length (nt)	SALSA MLPA probe	Gene / exon ^a	Location / ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
418	18796-L24994	KMT2E , ex 4	NM_182931.3; 626-627	TGTGGTAGTTGA-GAAATCCAACAG	15,2 kb
384	18528-L24355	KMT2E , ex 11	NM_182931.3; 1612-1613	AGTTTATGCTGA-GAGAACAGTTTG	43,9 Mb
308	18272-L23874	EZH2 , ex 14	NM_004456.5; 1761-1762	TGCCCTTGTGTG-ATAGCACAAAAT	17,7 kb
246	18267-L26041	EZH2 , ex 4	NM_004456.5; 483-484	TGGTCTCCCTA-CAGCAGAATTTT	-
Chromosome 11q alterations; ATM and KMT2A at 11q22.3-q23.3					
KMT2A (also known as MLL) alterations are observed in 5-20% of AML cases, typically comprising partial tandem duplications (PTDs), amplifications and translocations (Rao et al. 2015), and are associated with poor outcome (Choi et al. 2018; Issa et al. 2021; Yuen et al. 2023). KMT2A PTDs are most commonly duplications occurring within exon 2-10 of KMT2A gene and are associated with an unfavourable prognosis (Choi et al. 2018). Probes targeting PTDs are indicated by (+). KMT2A is flanked by UBE4A and TMEM25 genes.					
215 ~	06675-L06253	NUP98	11p15.4	CTTCCTTCGTAT-CTGGAGGGTTCT	16,5 Mb
296 ~	08936-L33053	SLC6A5	11p15.1	TGTTTGCCCTCT-TTGTGTCTGTAC	87,2 Mb
371	08420-L22087	ATM , ex 13	NM_000051.4; 2174-2175	AGAAAAGCACCA-GTCCAGTATTGG	18,9 kb
346	02663-L22101	ATM , ex 22	NM_000051.4; 3407-3406, reverse	CAGCCAACATGC-GAACTTGGTGAT	92,6 kb
290	08445-L23873	ATM , ex 63	NM_000051.4; 9192-9193	ATGAGACTACAA-GAGAACTGAAA	10,3 Mb
193 ~	23323-L32969	UBE4A , ex 18	NM_001204077.2; 2925-2926	CCAAGGATGGAC-GTTCTATTCCC	2,1 kb
251 ~	23325-L32971	UBE4A , ex 19	NM_001204077.2; 3064-3065	ACAGGAAGAGGA-AACCTATGCAGA	43,7 kb
KMT2A , at 11q23.3. Ligation site information for KMT2A is according to NM_001197104.2.					
130 «	23318-L32964	KMT2A , ex 1	4-3, reverse	CCCGTGAAGTGA-AGCAGCGAGAGG	0,5 kb
		Start codon	22-24		
358 «	23329-L32998	KMT2A , ex 1	92 nt after exon 1, reverse	GAGATGGTCCCA-GAATTGGGATGC	10,6 kb
303 Ø	23335-L32983	KMT2A , intr 1	10,7 kb after exon 1, reverse	TTCCAATCATAT-AATCATCTTTTG	21,1 kb
337 +	23327-L32973	KMT2A , ex 2	503-504	CAGAGTGCGAAG-TCCCACAAGGTC	3,5 kb
197 +	09499-L32954	KMT2A , ex 3	1220-1221	AAGAAAGGTGAA-GACACAGGTCAA	0,1 kb
443 +	19133-L32999	KMT2A , ex 3	1372-1373	CGAATAGTAGAT-TCAGTGCCCCGT	1,1 kb
321 +	23326-L32972	KMT2A , ex 3	2501-2502	GGCAGAGCCATT-TTCATCAAGTAG	3,2 kb
228 +	23334-L33002	KMT2A , ex 4	4 nt before exon 4, reverse	TCTTGACCCTGA-ATATGAGGCAAT	1,3 kb
280 +	19132-L25682	KMT2A , ex 5	3469-3468, reverse	CCTCGATCGACG-TCCTTTCTTTAC	2,1 kb
391 +	23330-L32996	KMT2A , ex 6	3623-3622, reverse	AGGCTTTGGAAG-GCATCCATTGTA	1,6 kb
145 +	23320-L32966	KMT2A , ex 7	3742-3743	TGGTGGAAGTCTA-GTCAGAAACCTA	2,4 kb
466 +	23332-L32978	KMT2A , ex 9	4150-4151	ATGCAGGCACTT-TGAACATCCTCA	0,7 kb
353 +	23328-L32974	KMT2A , ex 10	4336-4337	TTCTCTGTGCCA-GTAGTGGGCATG	5,2 kb
436	23331-L32977	KMT2A , ex 13	4636-4637	GCTGTGGATCCA-CAACTCCAGGCA	5,6 kb
484	23333-L32979	KMT2A , ex 19	5426-5427	ACCTTCACTTGA-CCATAATTATGC	16,2 kb
166	23321-L32967	KMT2A , ex 31	11114-11115	GTCATTGACAGA-TAAAGTCCAGGA	10,0 kb
186	19885-L29240	KMT2A , ex 36	11771-11772	GCCTAACTGCTA-TTCTCGGGTCAT	9,9 kb
		Stop codon	11938-11940		
136 ~	23319-L32965	TMEM25 , ex 2	NM_032780.4; 139-140	CTTCTGAGCTCA-GGTACACCCCTG	2,7 kb
177 ~	23322-L32968	TMEM25 , ex 9	NM_032780.4; 1160-1161	GCAGTGATGAGA-TCTGGCTCTGAG	9,5 Mb
182 ~	09496-L25025	ETS1	11q24.3	TGTGTATGCAAA-ATGAATGGCACA	-
Chromosome 17p and 17q deletions					
Deletion of 17p, harbouring TP53, is observed in around 5% of AML patients and can be present as a single aberration or in the context of a complex karyotype, the latter being associated with a poor outcome. Significant positive association between was identified between TP53 deletion and loss of chromosome 5, 5q deletion and loss of chromosome 7, all high-risk aberrations (Seifert et al. 2009; Walter et al. 2009). Moreover, loss of 17p arm and gain of 17q can result from isochromosome 17q formation (i(17q)) - an intermediate prognostic marker according to IPSS-R and IPSS-M (Greenberg et al. 2012; Bernard et al. 2022). Deletion of the tumour suppressor NF1 is observed in 4–11% of AML cases and may deregulate signalling pathways implicated in AML development and progression (Walter et al. 2009; Parkin et al. 2010; Boudry-Labis et al. 2013; Haferlach et al. 2009; Giraud et al. 2023). Another important target on 17q is SUZ12 (Score et al. 2012).					
The TP53 exon numbering is derived from MANE project according to NM_000546.6. The exon numbering used in previous versions of this product description can be found in between brackets.					

Length (nt)	SALSA MLPA probe	Gene / exon ^a	Location / ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
401	17423-L32981	TP53 , ex 9 (8)	1095-1096	CTCTCCCCAGCC-AAAGAAGAAACC	0,2 kb
139	08304-L26800	TP53 , ex 8 (7)	981-982	CTGTCCTGGGAG-AGACCGGCGCAC	1,4 kb
256	02376-L30912	TP53 , ex 5 (4b)	546-547	CAAGATGTTTTG-CCAACTGGCCAA	19,3 Mb
412	18544-L24995	NF1 , ex 34	NM_001042492.3; 4829-4830	TCTTTCCTTCAT-AAGTGACGGCAA	96,6 kb
154	12018-L32953	NF1 , ex 53	NM_001042492.3; 8120-8121	TTTACGTAAAGT-TTCAGTGTCTGA	631,4 kb
498	18539-L23830	SUZ12	17q11.2	CATCAGGAAAAC-AAGCCTGGTTCA	-
ASXL1 c.1934dupG mutation on 20q11.21 The ASXL1 c.1934dupG mutation is the most commonly-found genetic alteration in the ASXL1 gene in AML. ASXL1 loss-of-function mutations were shown to promote myeloid transformation, and are associated with a poor prognosis in AML and MDS (Abdel-Wahab et al. 2012; Gelsi-Boyer et al. 2012; Chou et al. 2010).					
160 § ± Ж	18261-SP0848-L26260	ASXL1 , ex 13	NM_015338.6, 2376-2375 and 2352-2351, reverse	CGGGCCACCCCC- 24nt spanning oligo- CCTCTCTATGGC	-

^a See section Exon numbering on page 2 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.

§ Mutation-specific probe. This probe will only generate a signal when the ASXL1 c.1934dupG mutation is present. It has been tested on artificial DNA and on DNA from the KASUMI-1 cell line.

± An unspecific peak might be detected at one nucleotide shorter length from the expected length of this ASXL1 c.1934dupG mutation-specific probe at 160 nt. Please analyse the peak pattern carefully when making calls for this mutation-specific probe.

« 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.

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.

- Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

+ This probe targets an exon in the KMT2A gene that is commonly affected by PTDs in AML.

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
315	14763-L24999	PPOX	1q23	AGACTGTGCACA-GTTTTGCCAGC	39,2 Mb
172	21100-L29489	CACNA1S	1q32	AAGCCGCCATGA-AGATCATTGCCT	-
148	17858-L23867	SLC3A1	2p21	CAGCCCAGATCG-GCTTTGAAGTTA	-
505	15203-L23403	GBE1	3p12	GACCTAGAGGGA-CTCATGATCTTT	75,0 Mb
489	17939-L15290	KCNAB1	3q25	CTTTTCAGAGA-GAGAAAGTGGAG	-
121	19616-L27455	ATP8A1	4p13	CAGATCTTCTT-CGAGGAGCTCAG	-
208	13384-L25019	EYS	6q12	ACAAGGGGTTGA-TACCATGTGGAC	-
126	S0959-L26042	PCSK5	9q21	AGAAAGGCCTGA-TCATGAACCCTC	-
407	01237-L24913	UPF2	10p14	TGCCATTCTTT-GCATCTCAAAAG	-
452	16286-L27983	RNASEH2B	13q14	TGACTGTTAATG-ACTACCTTTGGT	-
378	08141-L24356	RPGRIP1	14q11	TATTCCTTCTAT-GACTTTGAAACC	-
274	09792-L23872	SPG11	15q15	GCTGACAAGCAA-ATGCCTCCATGG	-
233	15154-L25342	MC4R	18q21	GACATTTACTCA-CAGCAGGCATGG	-

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

Related products

For related products, see the [product page](#) on our website.

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P496 product history	
Version	Modification
A1	First release.

Implemented changes in the product description
<p>Version A1-03 – 29 July 2025 (04P)</p> <ul style="list-style-type: none"> - Positive control DNA samples section: information moved to product page on website. - Removed Related SALSA MLPA products section. - Exon numbering of the <i>TP53</i> gene is now reported based on MANE Select Transcript NM_000546.6 in Table 2. <p>Version A1-02 – 29 November 2023 (04P)</p> <ul style="list-style-type: none"> - In the positive control sample DNA table on page 3 several corrections were made and for all the cell lines the ACC numbers were added in between brackets. - Minor typo corrections. <p>Version A1-01 - 14 November 2023 (04P)</p> <ul style="list-style-type: none"> - Not applicable, new document.

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