Product Description
SALSA® MLPA® Probemix P482-A1 DICER1

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 9.

Catalogue numbers:
- **P482-025R:** SALSA MLPA Probemix P482 DICER1, 25 reactions.
- **P482-050R:** SALSA MLPA Probemix P482 DICER1, 50 reactions.
- **P482-100R:** SALSA MLPA Probemix P482 DICER1, 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 P482 DICER1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *DICER1* gene, which is associated with DICER1 syndrome.

DICER1 gene locates on 14q32.13 (hg18), consists of 27 exons and encodes cytoplasmic endoribonuclease (RNase) III enzyme. DICER1 has a central role in the RNA interference pathway, as it cleaves double-stranded RNA molecules into small RNAs including microRNA (miRNA) and small interfering RNA (siRNA). DICER1 facilitates the incorporation of these RNAs into Argonoute protein, forming the RNA-induced silencing complex (RISC). The activated RISC recognizes a specific mRNA target sequence and can either guide degradation or inhibit translation of the molecule.

DICER1 syndrome, or *DICER1*-related pleuropulmonary blastoma cancer predisposition syndrome (OMIM 601200), is a rare pleiotropic tumour predisposition syndrome manifesting usually in children or young adults and it is characterised by benign and malignant tumours in lungs, ovaries, thyroid gland, kidneys and brain (pineal and pituitary glands). *DICER1* syndrome is inherited in an autosomal dominant pattern, but may also arise *de novo* in the germline or in a somatic mosaic manner. It is estimated that 80% of the germline pathogenic variants are inherited from a parent and 20% are *de novo* (see GeneReviews https://www.ncbi.nlm.nih.gov/books/NBK196157/). Most of the described mutations in *DICER1* are loss-of-function point or frameshift mutations, however, deletions of the entire *DICER1* locus and in- or out-of-frame intragenic *DICER1* deletions have also been identified (for review see De Kock et al. 2019).

Multinodular goiter-1 (MNG1) with or without Sertoli-Leydig cell tumours (OMIM 138800) is also caused by heterozygous mutations in the *DICER1* gene. Unlike *DICER1* syndrome, MNG1 is a common disorder characterized by nodular enlargement of the thyroid gland and some individuals may also develop Sertoli-Leydig cell tumours, usually of the ovary.

Additionally, mutations in *DICER1* are recurrent in diverse set of cancers e.g. in sporadic pleuropulmonary blastoma, gonadal-, Wilms' and endometrial tumours and anaplastic sarcomas of the kidney. Although

miRNAs are required for normal cell fitness, selective inactivation of *DICER1*, especially mutations in the RNase IIIb domain affecting the metal ion-binding residues, can benefit cancer cells (Vedanayagam et al. 2019).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK196157/ and González et al. 2022.

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): https://www.ncbi.nlm.nih.gov/refseq/MANE/ and http://tark.ensembl.org/
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *DICER1* exon numbering used in this P482-A1 DICER1 product description is the exon numbering from the MANE project (release version 1.0) based on MANE Select transcript NM_177438.3 as indicated in Table 2. 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 MANE sequence. 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 P482-A1 DICER1 contains 50 MLPA probes with amplification products between 124 and 500 nucleotides (nt). This includes 31 probes for the *DICER1* gene and four probes centromeric and three probes telomeric to *DICER1* gene. In addition, 12 reference probes are included that target relatively copy number stable regions in various cancer types. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and 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). 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 handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA, which includes DNA derived from paraffin-embedded tissues, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

Reference samples
A sufficient number (≥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 *DICER1* related disorders. 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. Samples described in the table below from the Coriell Institute and Leibniz Institute DSMZ have been tested with this P482-A1 probemix at MRC Holland and can be used as a positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P482-A1	Expected copy number alteration
NA13410	Coriell Institute	14q32.13	*DICER1(*and flanking genes *BTBD7, SERPINA3, GCS, CLMN, TCL1A, ATG2B)*	Heterozygous duplication
NA10074	Coriell Institute	14q32.13	*DICER1(*and flanking genes *RPGRIP1, BTBD7, SERPINA3, GCS, CLMN, TCL1A, ATG2B)*	Heterozygous duplication
L-363 ◊+ (ACC 49)	DSMZ	14q32.13	*DICER1 (*and flanking genes *BTBD7, SERPINA3, GCS, CLMN, TCL1A, ATG2B)*	Deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P482-A1 DICER1 probemix.

◊ Some of the reference probe targets are also affected by copy number alterations in this tumour cell line.

+ As this is a tumour cell line, reference probes are more prone to show deviating copy number results. Turning off the slope correction in Coffalyser.Net analysis can help to obtain the correct interpretation when regions targeted by reference probes are affected by their copy number.

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 / 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 mosaic or subclonal cases. Analysis of parental samples for germline analysis 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.

P482 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 most populations, the major cause of genetic defects in the *DICER1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P482 DICER1.
- 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 solid 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.

***DICER1* mutation database**

We strongly encourage users to deposit positive results in the LOVD (Leiden Open Variation Database) https://www.LOVD.nl/DICER1. 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 deletion of *DICER* exons 7 and 9 but not exon 8) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P482-A1 DICER1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			Location (hg18) in kb
		Reference	*DICER1*	Flanking	
64-105	Control fragments – see table in probemix content section for more information				
124	Reference probe 19616-L26275	4p13			04-042.278
130	Reference probe 13867-L15385	16p13			16-008.765
136 Ø	**DICER1 probe** 22501-L31650		Upstream		14-094.694
143	**DICER1 probe** 22504-L31653		Exon 18		14-094.642
148 ¬	**GSC probe** 22523-L31672			14q32.13	14-094.305
155	**DICER1 probe** 22507-L31656		Exon 11		14-094.652
160	**DICER1 probe** 22518-L31981		Exon 26		14-094.627
166	Reference probe 13816-L30646	2q13			02-108.906
170	**DICER1 probe** 22522-L31671		Exon 1		14-094.693
179	**DICER1 probe** 22511-L31660		Exon 16		14-094.644
185 ¬	**BTBD7 probe** 22490-L31982			14q32.13	14-092.800
190	**DICER1 probe** 22509-L31658		Exon 23		14-094.632
196	Reference probe 22483-L31204	21q22			21-037.920
202 Ø	**DICER1 probe** 22512-L31661		Intron 1		14-094.673
208	**DICER1 probe** 22492-L31983		Exon 8		14-094.661
215	**DICER1 probe** 22513-L31662		Exon 25		14-094.627
220	**DICER1 probe** 22515-L31984		Exon 2		14-094.670
227	Reference probe 22485-L09033	11p15			11-020.596
232	**DICER1 probe** 22514-L31663		Exon 13		14-094.648
238 ¬	**SERPINA3 probe** 22524-L31673			14q32.13	14-094.155
245	**DICER1 probe** 22508-L31657		Exon 27		14-094.627
250 Ø	**DICER1 probe** 22510-L31659		Intron 1		14-094.678
258	Reference probe 09064-L16631	19p13			19-013.302
266	**DICER1 probe** 22520-L31985		Exon 10		14-094.653
274	**DICER1 probe** 22521-L31670		Exon 4		14-094.668
283 ¬	**ATG2B probe** 22489-L31638			14q32.2	14-095.858
292	**DICER1 probe** 22517-L31666		Exon 17		14-094.644
301 Ø	**DICER1 probe** 22516-L31665		Intron 1		14-094.677
310	**DICER1 probe** 22519-L31668		Exon 20		14-094.641
320	Reference probe 14346-L16015	2q32			02-190.146
328 ¬	**RPGRIP1 probe** 22484-L20663			14q11.2	14-020.839
337	**DICER1 probe** 22496-L31645		Exon 9		14-094.654
346	**DICER1 probe** 22495-L31644		Exon 15		14-094.644
356	Reference probe 22486-L06313	15q24			15-070.425
365	**DICER1 probe** 22498-L31647		Exon 3		14-094.669
373	**DICER1 probe** 22497-L31986		Exon 24		14-094.630
383	**DICER1 probe** 22494-L31643		Exon 12		14-094.649
392	**DICER1 probe** 22493-L31642		Exon 6		14-094.666
400	Reference probe 14839-L16547	1p34			01-045.253
409	**DICER1 probe** 22505-L31654		Exon 22		14-094.636
418 « ¬	**CLMN probe** 22491-L31989			14q32.13	14-094.856
427	**DICER1 probe** 22506-L31655		Exon 14		14-094.647
436	**DICER1 probe** 22500-L31649		Exon 19		14-094.642
445	Reference probe 22487-L31637	10q21			10-055.296
454	**DICER1 probe** 22502-L31651		Exon 7		14-094.663
462	**DICER1 probe** 22503-L31987		Exon 21		14-094.640
472 ¬	**TCL1A probe** 22525-L31674			14q32.13	14-095.248
481	**DICER1 probe** 22499-L31988		Exon 5		14-094.666
495	Reference probe 22488-L24472	12q21			12-084.219
500	Reference probe 19555-L27674	2p13			02-071.750

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

« 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.

¬ 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.

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. *DICER1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Location (hg18) / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
328 ¬	22484-L20663	*RPGRIP1*	14q11.2	TCTATTTGTCCA-CAGGCTGAGGAC	72.0 **M**b
185 ¬	22490-L31982	*BTBD7*	14q32.13	CTCATCCATATG-GCTCTAAATGGG	1.4 **M**b
238 ¬	22524-L31673	*SERPINA3*	14q32.13	AAAAGTGGGTAA-TGGTGCCCATGA	149.8 kb
148 ¬	22523-L31672	*GSC*	14q32.13	AGGTTCTCGAGA-GCTTCGAGCTGC	321.5 kb
Centromeric to DICER1					
DICER1, at 14q32.13, ligation sites according to NM_177438.3 (unless otherwise noted)					
		stop codon	*6112-6114 (exon 27)*		
245 #	22508-L31657	**exon 27**	6034-6033 reverse	GGCAATCCTGTA-ACTTCGACCAAC	0.5 kb
160	22518-L31981	**exon 26**	5885-5884 reverse	GGGGTACATTTG-CAGAAAACTTTT	0.2 kb
215	22513-L31662	**exon 25**	5812-5813	TTTACATGGATA-GTGGGATGTCAC	2.7 kb
373	22497-L31986	**exon 24**	5619-5620	CACAAGTACTTC-AAAGCTGTCTCT	2.3 kb
190	22509-L31658	**exon 23**	4986-4987	ATTTCTTACGAC-TTGCACACTGAG	3.5 kb
409	22505-L31654	**exon 22**	4510-4511	ATGTAGTAAATC-AAGACAAAAGCA	4.1 kb
462	22503-L31987	**exon 21**	3814-3815	GCAGAACGTTGC-TCAGCGAGTCCC	1.3 kb
310	22519-L31668	**exon 20**	3501-3502	AGAAAAGCTGTT-TGTCTCCCCAGC	0.5 kb
436	22500-L31649	**exon 19**	3424-3425	AATGGGAAAGTC-TGCAGAATAAAC	0.5 kb
143	22504-L31653	**exon 18**	3193-3194	ATGTAGCTGATG-TGTACACTGATC	1.5 kb
292	22517-L31666	**exon 17**	3051-3050 reverse	CGAGCTTCAGAC-TTCTCAATATCT	0.3 kb
179	22511-L31660	**exon 16**	2847-2848	TTGAAGAAGTCT-GGTTTCATGTTG	0.3 kb
346	22495-L31644	**exon 15**	2752-2753	CAAGATGCTTTG-GAATACTGACGG	3.1 kb
427	22506-L31655	**exon 14**	2484-2485	GACCATTTGATG-CCAGTTGGGAAA	0.8 kb
232	22514-L31663	**exon 13**	2413-2414	GTGTACGATTGG-CTGAAAGAGTTG	0.9 kb
383	22494-L31643	**exon 12**	2319-2320	ACCCGAGAGTTG-CCTGATGGTACA	2.6 kb
155	22507-L31656	**exon 11**	2126-2127	TTCCAAGTCGGT-TGATACTGGTGA	0.8 kb
266	22520-L31985	**exon 10**	1983-1984	TATCGATCCTAT-GTTCAATCTAAA	1.1 kb
337	22496-L31645	**exon 9**	1801-1802	ATGGCATTGGGA-AGAATCAGCCTC	6.9 kb
208	22492-L31983	**exon 8**	1324-1325	GAATGATGGTAA-GAGAACTACAGA	2.1 kb
454	22502-L31651	**exon 7**	1134-1135	TTTACTGACAGA-AGTGGGCTTTAT	2.8 kb
392	22493-L31642	**exon 6**	1065-1066	GCAACTGACCTG-GTGGTCTTAGAC	0.6 kb
481	22499-L31988	**exon 5**	874-875	TGTTTGATGAGT-GTCATCTTGCAA	1.4 kb
274	22521-L31670	**exon 4**	744-743 reverse	CTCTCTTTTGTC-CAAGATGCATTT	1.0 kb
365	22498-L31647	**exon 3**	586-585 reverse	CTGATAGGACAG-CTCTTTAGTGAG	0.9 kb
220	22515-L31984	**exon 2**	354-355	TGAATGAAAAGC-CCTGCTTTGCAA	3.3 kb
		start codon	*346-348 (exon 2)*		
202 Ø	22512-L31661	**intron 1**	3.2 kb before exon 2 reverse (NM_001271282.3; 95-94 reverse)	AAGCAGTTGTCT-TTCTTTCCCTCT	4.0 kb
301 Ø	22516-L31665	**intron 1**	7.2 kb before exon 2 (NM_030621.4; 215-216)	GCAGTTCAGACA-AGAGCAACACAG	1.0 kb
250 Ø	22510-L31659	**intron 1**	8.2 kb before exon 2 (NM_030621.4; 111-112)	AATACAGACTTG-GAAACTCTGAAA	15.7 kb

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Location (hg18) / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
170	22522-L31671	**exon 1**	146-147	CCCAGGTGAATG-GAGTAACCTGAC	0.6 kb
136 Ø	22501-L31650	**upstream**	448 nt before exon 1 (NM_030621.4; 33-34)	TTCCCCAAATGC-GGAGGCCCCTGC	161.7 kb
Telomeric to DICER1					
418 « ¬	22491-L31989	*CLMN*	14q32.13	CCTTGCAGGTTC-TGCACTCGGATG	392.6 kb
472 ¬	22525-L31674	*TCL1A*	14q32.13	CGGTATCGTCCA-TCAGGGTAGAGC	609.9 kb
283 ¬	22489-L31638	*ATG2B*	14q32.2	TAGTGAGAATTG-CTGATTGCTTTA	-

[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.

« 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.

¬ 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's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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 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
400	14839-L16547	*UROD*	1p34	GGCTATGAGGTG-GTTGGGCTTGAC	01-045.253
500	19555-L27674	*DYSF*	2p13	CCATTGCCAAGA-AGGTCAGTGTCC	02-071.750
166	13816-L30646	*EDAR*	2q13	CTCACATTCCTT-GGTGTTGGGGGG	02-108.906
320	14346-L16015	*SLC40A1*	2q32	TCGCTGGTGGTA-CAGAATGTTTCA	02-190.146
124	19616-L26275	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042.278
445	22487-L31637	*PCDH15*	10q21	TGAGGAGATCCT-ACTTCAAGTTTC	10-055.296
227	22485-L09033	*SLC6A5*	11p15	TTTCTGCAGGGA-TTGAATATCCTG	11-020.596
495	22488-L24472	*ALX1*	12q21	ATGACACCTTAT-TCTCACTCGCCT	12-084.219
356	22486-L06313	*HEXA*	15q24	AGTTGACATCTG-ACCTGACATTTG	15-070.425
130	13867-L15385	*ABAT*	16p13	ACTTTGTGGAGA-AGCTCCGGCAGT	16-008.765
258	09064-L16631	*CACNA1A*	19p13	CACCATAAAGAA-AAGCAAGACAGA	19-013.302
196	22483-L31204	*KCNJ6*	21q22	AGCTCCTACATC-ACCAGTGAGATC	21-037.920

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

References

- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol.* 147:60-8.
- González IA et al. (2022). *DICER1* tumor predisposition syndrome: an evolving story initiated with the pleuropulmonary blastoma. *Mod Pathol.* 35:4-22.
- Hömig-Hölzel C and Savola. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol.* 21:189-206.
- De Kock L et al. (2019). Ten years of DICER1 mutations: Provenance, distribution, and associated phenotypes. *Hum Mutat*. 40:1939-53.
- 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.
- Vedanayagam J et al. (2019). Cancer-associated mutations in DICER1 RNase IIIa and IIIb domains exert similar effects on miRNA biogenesis. *Nat Commun*. 10:3682.

Selected publications using SALSA MLPA Probemix P482 DICER1

- Reschke M et al. (2021) Eye Tumors in Childhood as First Sign of Tumor Predisposition Syndromes: Insights from an Observational Study Conducted in Germany and Austria. *Cancers (Basel).* 13(8):1876.

P482 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-03 – 17 October 2023 (04P) - A typo (*SERPIN3 >SERPINA3*) corrected in *Positive control DNA samples* -table on page 3 and *GCS* gene added to the list of CNA affected flanking genes in all positive control DNA samples. Version A1-02 – 26 September 2023 (04P) - Product description adapted to a new template. - Added one new sample (NA10074) in the Positive samples paragraph on page 3. - Ligation sites of the probes targeting the *DICER1* gene updated in Table 2 according to new version of the NM_177438.3 reference sequence (MANE Select). Version A1-01— 10 December 2019 (02P) Not applicable, new document.

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