Product Description SALSA® MLPA® Probemix P238-B2 DNAH5

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, one reference probe has been removed. For complete product history see page 5.

Catalogue numbers:
- **P238-025R:** SALSA MLPA Probemix P238 DNAH5, 25 reactions.
- **P238-050R:** SALSA MLPA Probemix P238 DNAH5, 50 reactions.
- **P238-100R:** SALSA MLPA Probemix P238 DNAH5, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P238 DNAH5 is a **research use only (RUO)** assay for the detection of deletions or duplications in *DNAH5* gene, which is associated with primary ciliary dyskinesia (PCD).

PCD, also known as immotile ciliary syndrome or Kartagener Syndrome (KS), is characterized by dysfunction of motile cilia and flagella and affects about 1 in 10.000 to 20.000 individuals. Recurrent respiratory infections are caused by defective mucociliary clearance due to immotile or dysmotile respiratory cilia. Mutations in the *DNAH5* and *DNAI1* genes, on chromosomes 5p15.2 and 9p13.3 respectively, are found in 38% of PCD patients. For probes targeting exons of the *DNAI1* gene, SALSA MLPA Probemix P237 DNAI1 can be used.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1122/.

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/

Probemix content: The SALSA MLPA Probemix P238-B2 DNAH5 contains 37 MLPA probes with amplification products between 130 and 474 nt. This includes 22 probes for 22 out of 79 exons of the *DNAH5* gene. In addition, 15 reference probes are included and detect 14 different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.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, 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.mlpa.com.

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

No DNA controls results in only five major peaks shorter than 105 nt: four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 105 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

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

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: 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 unrelated individuals who are from families without a history of PCD. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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.mlpa.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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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 (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.

Limitations of the procedure:

- In most populations, another cause of genetic defects in the *DNAH5* gene are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemix P238 DNAH5.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.

Primary Ciliary Dyskinesia variant database: https://grenada.lumc.nl/LOVD2/CILD/home.php?select_db=DNAH5. 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 SNPs and unusual results to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P238-B2 DNAH5

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*DNAH5*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 19616-L26704	4p13	
137	**DNAH5 probe** 08035-L07816		**Exon 2**
142	Reference probe 19948-L27009	10q25	
148	**DNAH5 probe** 08047-L07828		**Exon 44**
160	**DNAH5 probe** 08037-L07818		**Exon 11**
166	Reference probe 03565-L02931	3p22	
172	**DNAH5 probe** 08050-L07831		**Exon 55**
184	Reference probe 16364-L18757	12q13	
196	**DNAH5 probe** 08057-L07838		**Exon 79**
202	**DNAH5 probe** 08038-L07819		**Exon 14**
214	**DNAH5 probe** 08049-L07830		**Exon 52**
219	Reference probe 02608-L02079	11p11	
226	**DNAH5 probe** 08042-L07823		**Exon 29**
232	**DNAH5 probe** 08056-L07837		**Exon 78**
238	Reference probe 17365-L21549	20q13	
244	**DNAH5 probe** 08051-L07832		**Exon 59**
252	**DNAH5 probe** 08043-L07824		**Exon 31**
265	Reference probe 02125-L01636	13q21	
274	**DNAH5 probe** 08053-L07834		**Exon 66**
283	**DNAH5 probe** 08044-L07825		**Exon 34**
292	Reference probe 20529-L28119	1q31	
301	**DNAH5 probe** 08036-L07817		**Exon 4**
310	Reference probe 01293-L00838	9p21	
319	**DNAH5 probe** 08048-L07829		**Exon 48**
328	Reference probe 15984-L18139	18q21	
337	**DNAH5 probe** 08040-L07821		**Exon 20**
355	**DNAH5 probe** 08052-L07833		**Exon 62**
391	**DNAH5 probe** 08054-L07835		**Exon 70**
400	Reference probe 03004-L02443	19p13	
409	**DNAH5 probe** 08034-L07815		**Exon 1**
418	Reference probe 04792-L04167	9q34	
427	**DNAH5 probe** 08046-L07827		**Exon 40**
436	Reference probe 13809-L15303	5q14	
445	**DNAH5 probe** 08045-L07826		**Exon 37**
454	Reference probe 12759-L13875	17p13	
463	**DNAH5 probe** 08055-L07836		**Exon 74**
474	Reference probe 14956-L16689	6q22	

Note: The exon numbering used in this P238-B2 DNAH5 product description is the exon numbering from the RefSeq transcript NM_001369.2. The exon numbering and NM sequence used is from 06/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. *DNAH5* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*DNAH5* exon	Ligation site NM_001369.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*43-45 (Exon 1)*		
409	08034-L07815	Exon 1	48-49	GCTACAATGTTT-AGGATTGGGAGG	13.3 kb
137	08035-L07816	Exon 2	198-199	GACCTGAACAAA-ACCGAAGTGGAG	7.8 kb
301	08036-L07817	Exon 4	427-428	GGGTATGTGTGT-TCTTCATCAGGA	9.5 kb
160	08037-L07818	Exon 11	1494-1495	GCCAAGATAATA-GACATCTTTACA	12.4 kb
202	08038-L07819	Exon 14	1897-1898	CTCTGGCTCGAA-ACCAGCCTCCCA	18.5 kb
337	08040-L07821	Exon 20	3148-3149	AGACCCTGAACA-AAGCCGTGGAGT	20.3 kb
226	08042-L07823	Exon 29	4704-4705	GTGATTAATGAA-TGGGACAATAAA	12.0 kb
252	08043-L07824	Exon 31	5082-5083	CAGTGCTGTGTT-GGAGATGAGACC	9.7 kb
283	08044-L07825	Exon 34	5654-5655	CAATACATTGAT-AGACGTCACCAC	10.9 kb
445	08045-L07826	Exon 37	6174-6175	GTTCTCTCGGTT-GCAGCCCAGCAA	6.8 kb
427	08046-L07827	Exon 40	6696-6697	GACAAGGCAGGT-TACCCTGAACTG	11.5 kb
148	08047-L07828	Exon 44	7336-7337	ACACCGAGTCTT-TCCCAGACTTGT	17.8 kb
319	08048-L07829	Exon 48	8005-8006	AGATGACTGTTT-TTATTGATGATG	7.7 kb
214	08049-L07830	Exon 52	8730-8731	ACACCTAAAATT-TATGAGCCAATT	9.7 kb
172	08050-L07831	Exon 55	9262-9263	TGCACGACTACT-TCATGAGTCGGG	10.5 kb
244	08051-L07832	Exon 59	10058-10059	TGCTGTGAAAAT-TGACCTGGAAAA	11.8 kb
355	08052-L07833	Exon 62	10541-10542	GGCAGGTGAAAA-AGAAAGATGGAC	17.0 kb
274	08053-L07834	Exon 66	11445-11446	AAGCTAGAAATT-TCTGCTGAGACA	9.7 kb
391	08054-L07835	Exon 70	11959-11960	GGAAAATTTGGT-TTGATAAGGAAA	11.0 kb
463	08055-L07836	Exon 74	12789-12790	ATAGGAGAGATT-CAATATGGAGGC	15.8 kb
232	08056-L07837	Exon 78	13587-13588	GTGCTTTGCAAT-GAAGTCACCAAA	8.7 kb
196	08057-L07838	Exon 79	13828-13829	GAACGGACTTGA-ACTACATTGCCG	
		stop codon	*13915-13917 (Exon 79)*		

Note: The exon numbering used in this P238-B2 DNAH5 product description is the exon numbering from the RefSeq transcript NM_001369.2. The exon numbering and NM sequence used is from 06/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

P237 DNAI1 Contains probes for the *DNAI1* gene.

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.

P238 Product history	
Version	*Modification*
B2	One reference probe has been removed.
B1	One target probe and one reference probe have been removed and nine reference probes have been replaced.
A3	Two reference probes have been removed and QDX2 fragments have been added.
A2	Control fragment at 118 nt has been removed and one reference probe has been added.
A1	First release.

Implemented changes in the product description

Version B2-01 – 03 July 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2).

Version 09 (55) - 14 June 2016
- Product description adapted to a new version (lot number added, new picture included, changes to table 1 and table 2).

Version 08 (53)
- Warning added under Table 1, 346 nt probe 01335-L00879.

Version 07 (48)
- Warning added under Table 1, 142 nt probe 04712-L04130.

Version 06 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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