Product Description SALSA® MLPA® Probemix P237-B2 DNAI1

To be used with the MLPA General Protocol.

Version B2. As compared to B1, four reference probes have been replaced and one probe length has been adjusted. For complete product history see page 5.

Catalogue numbers:
- **P237-025R:** SALSA MLPA Probemix P237 DNAI1, 25 reactions.
- **P237-050R:** SALSA MLPA Probemix P237 DNAI1, 50 reactions.
- **P237-100R:** SALSA MLPA Probemix P237 DNAI1, 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.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 P237 DNAI1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *DNAI1* gene, which is associated with primary ciliary dyskinesia (PCD).

PCD phenotype is estimated to affect 1 in 10,000 to 20,000 individuals. PCD is characterised by dysfunction of motile cilia and flagella. Recurrent respiratory infections are caused by defective mucociliary clearance due to immotile or dysmotile respiratory cilia. Mutations in several genes have been implicated in PCD, including *DNAI1* in 2-10% of the cases.

More information is available at http://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/

Exon numbering: The *DNAI1* exon numbering used in this P237-B2 DNAI1 product description is the exon numbering from the RefSeq transcript NM_012144.4, which is identical to the NG_008127.1 sequence. The exon numbering and NM_ sequence used have been retrieved on 03/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P237-B2 DNAI1 contains 30 MLPA probes with amplification products between 131 and 463 nucleotides (nt). This includes 20 probes for the *DNAI1* gene, one probe for each exon of the gene. In addition, ten 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.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, 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.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 nt and 96 nt fragment 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.mlpa.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 unrelated individuals who are from families without a history of primary ciliary dyskinesia. 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 Institute (https://catalog.coriell.org) and Leibniz Institute 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 each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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 (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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *DNAI1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P237 DNAI1.
- 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.

***DNAI1* mutation database:** https://databases.lovd.nl/shared/genes/DNAI1. 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 (e.g., a duplication of *DNAI1* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P237-B2 DNAI1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*DNAI1*
64-105	Control fragments – see table in probemix content section for more information		
131 ¥	Reference probe 00797-L25925	5q31	
142	**DNAI1 probe** 08073-L07854		**Exon 15**
154	**DNAI1 probe** 08060-L07841		**Exon 2**
166 «	**DNAI1 probe** 08077-L07858		**Exon 19**
172	Reference probe 04731-L04148	7q21	
178	**DNAI1 probe** 08072-L07853		**Exon 14**
189	**DNAI1 probe** 08067-L07848		**Exon 9**
208 «	**DNAI1 probe** 08078-L07859		**Exon 20**
220	**DNAI1 probe** 08064-L07845		**Exon 6**
229 *	Reference probe 16553-L19044	11q13	
238	**DNAI1 probe** 08070-L07851		**Exon 12**
250	**DNAI1 probe** 08065-L07846		**Exon 7**
256	Reference probe 05911-L23142	21q11	
265	**DNAI1 probe** 08063-L07844		**Exon 5**
283	**DNAI1 probe** 12635-L07847		**Exon 8**
292	**DNAI1 probe** 08062-L07843		**Exon 4**
304	Reference probe 16436-L18889	18q21	
310	**DNAI1 probe** 08068-L23143		**Exon 10**
328	**DNAI1 probe** 08075-L07856		**Exon 17**
337	Reference probe 03264-L02701	3q29	
346	**DNAI1 probe** 08069-L07850		**Exon 11**
364	**DNAI1 probe** 08061-L07842		**Exon 3**
373 *	Reference probe 18340-L23253	6q24	
382	**DNAI1 probe** 08071-L07852		**Exon 13**
409 *	Reference probe 16283-L18575	13q14	
418	**DNAI1 probe** 08074-L07855		**Exon 16**
427	Reference probe 04444-L03830	8p21	
436 «	**DNAI1 probe** 08076-L07857		**Exon 18**
454 «	**DNAI1 probe** 08059-L07840		**Exon 1**
463 *	Reference probe 16106-L18276	17q21	

a) See above section on exon numbering for more information.

* New in version B2.
¥ Changed in version B2. Minor alteration, no change in sequence detected.
« 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.

Table 2. *DNAI1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*DNAI1* exon[a]	Ligation site NM_012144.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*202-204 (Exon 1)*		
454 «	08059-L07840	Exon 1	109-110	ACTGAAGTGGAA-GAGAGTCCAGAT	24.6 kb
154	08060-L07841	Exon 2	270-271	ATAGGCAGAGGA-ACCAGGAAGAGA	1.7 kb
364	08061-L07842	Exon 3	338-339	GGCCCAATCCAA-AGCCACAGTTAG	0.3 kb
292	08062-L07843	Exon 4	408-409	TTCACTCGGATT-TTGACAGCCAAC	3.9 kb
265	08063-L07844	Exon 5	507-508	CAACTGGCAGTT-CACTACACCCAG	0.7 kb
220	08064-L07845	Exon 6	619-620	AGGTGATTTCAG-AAACAGGAAACC	0.4 kb
250	08065-L07846	Exon 7	732-733	GAAGAAGAATTG-ATGACTCCTAAG	1.1 kb
283	12635-L07847	Exon 8	864-865	AGGACAAACTTT-TCAGCCACAGCC	1.7 kb
189	08067-L07848	Exon 9	938-939	AAAGACCAAAGA-GAAGGAGAAGGC	3.9 kb
310	08068-L23143	Exon 10	1052-1053	CCAAGCTGCTAA-GATCATGGAGCG	3.6 kb
346	08069-L07850	Exon 11	1104-1105	TGTGTTTAAGAT-TTTAAGTACTAT	0.4 kb
238	08070-L07851	Exon 12	1245-1246	AGGGATCTGTTT-GCAGTGGGATAT	5.6 kb
382	08071-L07852	Exon 13	1370-1371	CATGTGTCTCGA-CATCCACGTGGA	5.4 kb
178	08072-L07853	Exon 14	1542-1543	GACATGGACCAA-AACCTTAACTTC	0.2 kb
142	08073-L07854	Exon 15	1614-1615	AGAAAGCTGGTT-CACATAGATGTC	0.8 kb
418	08074-L07855	Exon 16	1706-1707	TGGCACTGCCTT-TGACTTCCACAA	1.3 kb
328	08075-L07856	Exon 17	1795-1796	ACTCCAGCCAAT-TCCTCGACACCT	0.3 kb
436 «	08076-L07857	Exon 18	1993-1994	CTTCTACTGTGT-TCGCAGCAGTCA	2.6 kb
166 «	08077-L07858	Exon 19	2092-2093	CCAAAAAGAACA-GGCTCACCCACG	3.4 kb
208 «	08078-L07859	Exon 20	2269-2270	ACAAACTGCTGA-ACCTGGTGAGGG	
		stop codon	*2299-2301 (Exon 20)*		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.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.

Related SALSA MLPA probemixes

P238 DNAH5 Contains probes for the *DNAH5* 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.

Selected publications using SALSA MLPA Probemix P237 DNAI1

- Ziętkiewicz E et al. (2010). Population specificity of the DNAI1 gene mutation spectrum in primary ciliary dyskinesia (PCD). *Respir Res*, 11(1), 174.

P237 Product history	
Version	*Modification*
B2	Four reference probes have been replaced and one reference probe length has been adjusted.
B1	Two reference probes have been replaced and six have been excluded from the mix. Furthermore the control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description
Version B2-01 – 26 March 2020 (02P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *DNAI1* gene updated according to new version of the NM_ reference sequence. *Version 08 – 14 July 2016 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Ligation sites updated according to new version of the NM_reference sequenced (Table 2). - Data analysis method has been modified. - Various minor textual changes. *Version 07 (49)* - Electropherogram picture of old buffer (introduced Dec. 2012) removed. *Version 06 (49)* - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included). *Version 05 (48)* - Warning added in Table 1, 256 nt probe 03785-L03294. *Version 04 (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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