SALSA MLPA probemix P076-B2 ACADVL-SLC22A5

Lot B2-1217. As compared to version B1 (B1-0714), three reference probes have been replaced and one probe length has been adjusted.

Very Long-Chain Acyl-coenzyme A Dehydrogenase (VLCAD) deficiency (OMIM 201475) is a fatty acid oxidation disorder that is detected in newborn screening. VLCAD is a metabolic disorder which prevents the converting of certain fats to energy. Defects of the ACADVL (acyl-Coenzyme A dehydrogenase, very long chain) gene are the cause of VLCAD deficiency; 80% of the cases of VLCAD deficiency are caused by mutations in the ACADVL gene, 20% of the cases are caused by complete or partial deletions of ACADVL.

Another fatty acid oxidation disorder is primary carnitine deficiency (OMIM 212140). Most cases of primary carnitine deficiency are caused by mutations in the SLC22A5 gene (solute carrier family 22 member 5), however, 20% of the cases do not show any mutation and might be caused by (partial) deletions of SLC22A5.

The ACADVL gene (20 exons) spans ~5.4 kb of genomic DNA and is located on chromosome 17p13.1, 7 Mb from the p-telomere. The SLC22A5 gene (10 exons) spans ~26 kb of genomic DNA and is located on chromosome 5q31.1, 132 Mb from the p-telomere.

The P076-B2 ACADVL-SLC22A5 probemix contains one probe for each exon of the ACADVL gene, except for exon 2. Two probes are present for exon 4. This probemix also contains one probe for each exon of the SLC22A5 gene. In addition, 12 reference probes are included in this probemix, detecting several different autosomal chromosomal locations.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned genes in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak height, even when not located exactly on the ligation site! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic; users should always verify the latest scientific literature when interpreting their findings. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Finally, note that most defects in this gene are expected to be small (point) mutations which will not be detected by this SALSA® MLPA® test.

SALSA® MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MLPA® test probemixes and reagents includes a limited license to use these products for research purposes.

The use of an SALSA® MLPA® probemix and reagents requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002).

Related SALSA® MLPA® probemixes

- P465 ACADM: Contains probes for the ACADM gene, involved in Medium-Chain Acyl-Coenzyme A Dehydrogenase (MCAD) Deficiency.

More information

Website : www.mlpa.com; www.mlpa.eu
E-mail : info@mlpa.com (information & technical questions); order@mlpa.com (for orders)
Mail : MRC-Holland bv; Willem Schoutenstraat 1, 1057 DL Amsterdam, the Netherlands

References

- Frigeni M, et al., 2017. Functional and molecular studies in primary carnitine deficiency. *Hum Mutat* 38.12: 1684-1699.

Data analysis

The P076-B2 ACADVL-SLC22A5 probemix contains 42 MLPA probes with amplification products between 130 and 503 nt. In addition, it contains nine control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA Denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and one Y-fragment at 105 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Data generated by this probemix can first be normalised intra-sample by dividing the peak height of each probe's amplification product by the total peak height of only the reference probes in this probemix (block normalisation). Secondly, inter-sample normalisation can be achieved by dividing the intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes no changes occurred in the genomic regions recognised by the reference probes.

Data normalisation should be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blotting, long range PCR, qPCR, FISH.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

Many copy number alterations in healthy individuals are described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. For example, a duplication of a complete gene might not be pathogenic, while a partial duplication or a deletion may result in disease. For some genes, certain in-frame deletions may result in a very mild, or no disease. Copy number changes of reference probes are unlikely to be the cause of the condition tested for. Users should always verify the latest scientific literature when interpreting their findings.

This probemix was developed at MRC-Holland.

Info/remarks/suggestions for improvement: info@mlpa.com.

Table 1. SALSA MLPA P076-B2 ACADVL-SLC22A5 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position		
		reference	ACADVL	SLC22A5
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA			
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation			
100	X-fragment: Specific for the X chromosome			
105	Y-fragment: Specific for the Y chromosome			
130 *	Reference probe 08640-L08656	3q26		
136	**ACADVL probe** 13445-L14900		**Exon 1**	
142	Reference probe 07721-L07431	7p13		
148	**SLC22A5 probe** 15914-L18032			**Exon 9**
157	**ACADVL probe** 13447-L19445		**Exon 10**	
166	**SLC22A5 probe** 15915-L18033			**Exon 7**
172	**ACADVL probe** 13443-L14898		**Exon 14**	
178	**SLC22A5 probe** 15916-L18034			**Exon 4**
190	Reference probe 12422-L13423	14q24		
196	**ACADVL probe** 13444-L14899		**Exon 9**	
202	**ACADVL probe** 13449-L14904		**Exon 17**	
209 Ж	**SLC22A5 probe** 15917-SP0306-L18035			**Exon 3**
220	Reference probe 12427-L13428	22q12		
229	**SLC22A5 probe** 15918-L18036			**Exon 2**
238	Reference probe 01640-L01178	11q23		
252	**SLC22A5 probe** 15919-L18037			**Exon 8**
265	**ACADVL probe** 13458-L14913		**Exon 7**	
273 *	Reference probe 15957-L28149	6q15		
281	**ACADVL probe** 13452-L14907		**Exon 16**	
292	**ACADVL probe** 13454-L14909		**Exon 6**	
301	**ACADVL probe** 13434-L14889		**Exon 20**	
310	**SLC22A5 probe** 15920-L18038			**Exon 6**
319 Ж	**ACADVL probe** 16854-SP0400-L19648		**Exon 8**	
328 *	Reference probe 17044-L20107	10p11		
337	**SLC22A5 probe** 16845-L19635			**Exon 5**
346	**ACADVL probe** 13441-L14896		**Exon 19**	
355	**ACADVL probe** 13442-L15597		**Exon 11**	
364 ¥	**ACADVL probe** 21571-L30567		**Exon 12**	
372 ±	**SLC22A5 probe** 15922-L18040			**Exon 1**
382	Reference probe 10140-L10602	18q11		
391	**ACADVL probe** 13453-L14908		**Exon 4**	
400	**ACADVL probe** 13461-L14916		**Exon 15**	
409	Reference probe 09999-L10331	20q13		
419	**ACADVL probe** 13456-L14911		**Exon 5**	
427	Reference probe 04444-L03830	8p21		
445	**ACADVL probe** 16855-L19649		**Exon 3**	
454	**ACADVL probe** 13437-L14892		**Exon 18**	
463	**ACADVL probe** 13436-L14891		**Exon 13**	
472	Reference probe 12761-L13877	4q12		
483	**ACADVL probe** 13439-L14894		**Exon 4**	
492	**SLC22A5 probe** 15923-L18041			**Exon 10**
503	Reference probe 09870-L18172	2p15		

* New in version B2 (from lot B2-1217 onwards).
¥ Changed in version B2 (from lot B2-1217 onwards). Small change in length, no change in sequence detected.
Ж This probe consists of three parts and have two ligation sites. This type of probe is more sensitive to depurination, for instance, when buffer capacity is insufficient.
± This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

Note: Exon numbering used here may differ from literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. P076 probes arranged according to chromosomal location

Table 2a. ACADVL gene

Length (nt)	SALSA MLPA probe	ACADVL exon	Ligation site in NM_000018.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*155-157 (exon 1)*		
136	13445-L14900	Exon 1	148-147 reverse	TGCATCTCCGAA-TCTCTCCGGGCG	0.4 kb
	No probe	Exon 2			
445	16855-L19649	Exon 3	32 nt before exon 3 reverse	CAAGTTCAGGGA-AGGGACTTCCGC	0.1 kb
391	13453-L14908	Exon 4	31 nt before exon 4 reverse	TTTCAGGGCTCT-GGTTGGGTCTGG	0.1 kb
483	13439-L14894	Exon 4	408-409	GCTCACCACAGA-TCAGGTGTTCCC	0.2 kb
419	13456-L14911	Exon 5	488-489	CTGTGTCCCGTT-TCTTCGAGGTAA	0.1 kb
292	13454-L14909	Exon 6	518-519	ATCCCGCCAAGA-ATGACGCTCTGG	0.6 kb
265	13458-L14913	Exon 7	664-665	GTGGGCATGCAT-GACCTTGGCGTG	0.5 kb
319 Ж	16854-SP0400-L19648	Exon 8	4 nt and 31 nt after exon 8	TGGATCAGGCAA-27nt spanning oligo-CCGCCCAATTCC	0.1 kb
196	13444-L14899	Exon 9	927-926 reverse	AGACCGTGAAGA-TGTCTGCTAGGC	0.5 kb
157	13447-L19445	Exon 10	1080-1081	AAACACAGCAGA-GGTGTTCTTTGA	0.5 kb
355	13442-L15597	Exon 11	1310-1311	AGCTGGCACGGA-TGGTTATGCTGC	0.4 kb
364	21571-L30567	Exon 12	2 nt before exon 12	CTATGCAACCTC-AGTCCATGGCTT	0.2 kb
463	13436-L14891	Exon 13	13 nt before exon 13	AGTCTCATCTGT-TCTTTGTCCCTA	0.2 kb
172	13443-L14898	Exon 14	1550-1549 reverse	CCGAAGAATGTC-ATTTGTCCCCTC	0.1 kb
400	13461-L14916	Exon 15	1594-1593 reverse	AGCTCCTTTCCT-TTGTCCTATGGG	0.2 kb
281	13452-L14907	Exon 16	19 nt before exon 16	ACTAACCAGTCA-TTCTCCCTCTTC	0.2 kb
202	13449-L14904	Exon 17	1810-1809 reverse	TTCTTGTGTTTT-ATCAGCTTGGCC	0.2 kb
454	13437-L14892	Exon 18	1902-1901 reverse	CTCCTCACCTCG-AGAGAACCACCA	0.1 kb
346	13441-L14896	Exon 19	1955-1954 reverse	GTCACAGAGCAT-TTTCTCATGCTG	0.2 kb
301	13434-L14889	Exon 20	2064-2065	CTTCAAAAGCAT-CTCCAAGGCCTT	
		stop codon	*2120-2122 (exon 20)*		

Ж This probe consists of three parts and has two ligation sites. This type of probe is more sensitive to depurination, for instance, when buffer capacity is insufficient.

The NCBI NM_000018.3 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Table 2b. SLC22A5 gene

Length (nt)	SALSA MLPA probe	SLC22A5 Exon	Ligation site in NM_003060.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*222-224 (exon 1)*		
372 ±	15922-L18040	Exon 1	348-349	CCGTGTTCCTGA-TAGCGACCCCGG	8.3 kb
229	15918-L18036	Exon 2	684-683, reverse	GAAGGAGCCCAA-CAGCACACCCAC	5.7 kb
209 Ж	15917-SP0306-L18035	Exon 3	734-733; 11 nt before exon 3 reverse	ACGAACAGCACA-26nt spanning oligo-AGGAGAGTGACA	1.2 kb
178 #	15916-L18034	Exon 4	905-906	TCAGTTCGTATA-ATATTCTCTACG	1.7 kb
337	16845-L19635	Exon 5	1093-1094	GGGACGATTTGA-AGAGGCAGAGGT	1.9 kb
310	15920-L18038	Exon 6	1229-1230	GATCTGCTTCGA-ACCTGGAATATC	1.8 kb
166	15915-L18033	Exon 7	1336-1335 reverse	AGTTCACAAAGA-TGTCCCCATGCA	1.7 kb
252	15919-L18037	Exon 8	1510-1509 reverse	CCATCACCAGGA-CTGTAGCCAAAT	1.3 kb
148	15914-L18032	Exon 9	1709-1710	ATTCTCATGGGA-AGTCTGACCATC	1.2 kb
492	15923-L18041	Exon 10	2506-2507	TGTGAGCTCTTA-AGACCACTCAGC	
		stop codon	*1893-1895 (exon 10)*		

Ж This probe consists of three parts and has two ligation sites. This type of probe is more sensitive to depurination, for instance, when buffer capacity is insufficient.
± This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
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.

Note: Exon numbering used here may differ from to literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P076-B2 ACADVL-SLC22A5 sample picture

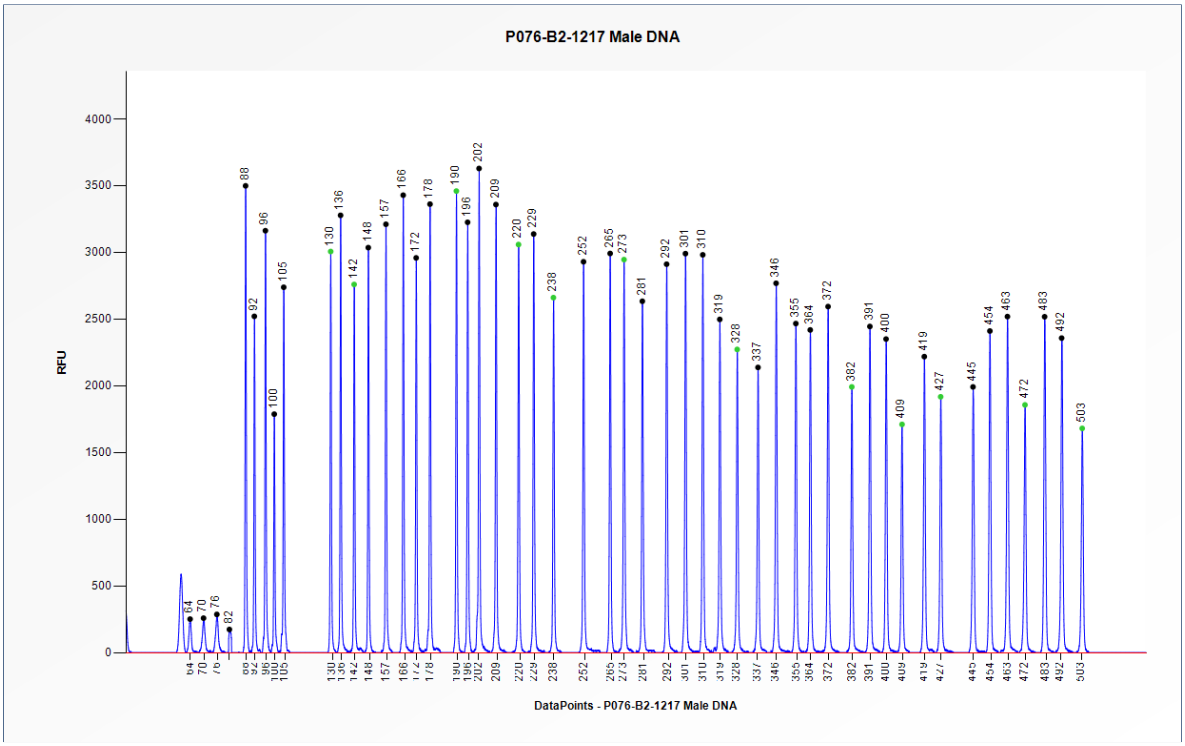

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA® MLPA® probemix P076-B2 ACADVL-SLC22A5 (lot B2-1217).

Implemented Changes – compared to the previous product description versions.
Version 08 – 07 March 2018 (55) - 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). - Related probemix added on page 1. - New reference added on page 2. - Ligation sites of the probes targeting the SLC22A5 gene updated according to NM_003060.2 sequence which is equal to NG_008982.1. *Version 07 – 26 November 2015 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Various minor textual changes throughout the document. - Ligation sites of the probes targeting the ACADVL gene updated according to new version of the NM_ reference sequence. *Version 06 – 12 August 2015 (54)* - Various minor textual changes. - Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed. - "Peak area" replaced with "peak height". *Version 05 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 04 (48)* - Remark on RefSeqGene standard and transcript variant added below Table 2. - Minor textual changes. *Version 03 (46)*

- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Minor textual changes.

Version 02 (46)

- Warning added: SLC22A5 probes are more variable.
- Sentence "when only small numbers of samples are tested, visual comparison of peak profiles should be sufficient" removed from data analysis section.

Version 01(46)

- Not applicable, new document.