Product Description SALSA® MLPA® Probemix P397-A3 SCN4A-CACNA1S

To be used with the MLPA General Protocol.

Version A3. Compared to version A2, one reference probe has been removed, three reference probes have been replaced, and one reference probe length has been adjusted. For complete product history see page 7.

Catalogue numbers:
- **P397-025R:** SALSA MLPA Probemix P397 SCN4A-CACNA1S, 25 reactions.
- **P397-050R:** SALSA MLPA Probemix P397 SCN4A-CACNA1S, 50 reactions.
- **P397-100R:** SALSA MLPA Probemix P397 SCN4A-CACNA1S, 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 P397 SCN4A-CACNA1S is a **research use only (RUO)** assay for the detection of deletions or duplications in the *CACNA1S* and *SCN4A* genes, which are associated with hypokalemic periodic paralysis.

The nondystrophic myotonias and primary periodic paralyses are an important group of genetic muscle diseases characterised by dysfunction of ion channels that regulate membrane excitability. Clinical manifestations vary and include myotonia, hyperkalemic, hypokalemic periodic paralysis, progressive myopathy, and cardiac arrhythmias.

Hypokalemic periodic paralysis (HypokalemicPP) is most commonly associated with mutations in *CACNA1S* gene (type 1 HypokalemicPP), which encodes the α subunit of the voltage-gated calcium channel, Cav1.1 (also known as the skeletal muscle L-type calcium channel, and the dihydropyridine receptor). Cav1.1 in the T-tubular membrane is attached to the ryanodine receptor of the sarcoplasmic reticulum, for which it acts as a voltage sensor. About 10% of hypokalemic periodic paralysis is associated with mutations in *SCN4A* gene (type 2 HypokalemicPP), which encodes the skeletal muscle sodium channel.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1338/.

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 *SCN4A* and *CACNA1S* exon numbering used in this P397-A3 SCN4A-CACNA1S product description is the exon numbering from the RefSeq transcripts NM_000334.4 and NM_000069.3, which are identical to the NG_011699.1 and NG_009816.2 sequences respectively. 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 P397-A3 SCN4A-CACNA1S contains 43 MLPA probes with amplification products between 131 and 490 nucleotides (nt). This includes 13 probes for the *CACNA1S* gene, targeting 13 out of 44 exons of the gene, and 19 probes for the *SCN4A* gene, targeting 19 out of 24 exons of the gene. In addition, eleven 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 hypokalemic periodic paralysis. 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 *CACNA1S* and *SCN4A* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P397 SCN4A-CACNA1S.
- 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.

***SCN4A* and *CACNA1S* mutation database:** https://databases.lovd.nl/shared/genes/. 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 *SCN4A* exons 2 and 4 but not exon 3) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P397-A3 SCN4A-CACNA1S

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	CACNA1S	SCN4A
64-105	Control fragments – see table in probemix content section for more information			
131 ¥	Reference probe 00797-L25925	5q31		
136	**SCN4A probe** 16602-L19132			**Exon 8**
141	**SCN4A probe** 16603-L19133			**Exon 1**
148	**SCN4A probe** 16604-L19134			**Exon 13**
154	Reference probe 17907-L22212	8p21		
160	**SCN4A probe** 16606-L19136			**Exon 18**
166	**SCN4A probe** 16607-L19137			**Exon 12**
172	**CACNA1S probe** 21100-L29489		**Exon 3**	
177	Reference probe 04359-L03779	7p13		
184	**CACNA1S probe** 16608-L19138		**Exon 20**	
196	**SCN4A probe** 16610-L19140			**Exon 4**
202	**CACNA1S probe** 16611-L19141		**Exon 8**	
208	**CACNA1S probe** 16612-L19142		**Exon 33**	
214 *	Reference probe 21316-L29722	3p14		
221	**SCN4A probe** 16613-L19143			**Exon 9**
226	**CACNA1S probe** 16614-L21583		**Exon 12**	
234	**SCN4A probe** 16615-L22853			**Exon 10**
238	**CACNA1S probe** 16616-L19146		**Exon 1**	
244	**SCN4A probe** 16617-L19147			**Exon 20**
256	**SCN4A probe** 16618-L19148			**Exon 6**
265	Reference probe 02454-L01898	15q21		
274	**SCN4A probe** 16619-L19149			**Exon 23**
282	**SCN4A probe** 16620-L19150			**Exon 16**
288	**SCN4A probe** 16622-L21584			**Exon 14**
296	**CACNA1S probe** 16621-L21585		**Exon 35**	
303	Reference probe 03242-L19700	13q14		
319	**SCN4A probe** 16623-L19153			**Exon 21**
328	Reference probe 02833-L02262	21q22		
335	**SCN4A probe** 16624-L19154			**Exon 19**
346	Reference probe 09722-L10072	12q24		
355	**SCN4A probe** 17933-L22239			**Exon 24**
364	**CACNA1S probe** 16627-L19157		**Exon 5**	
372	**CACNA1S probe** 16628-L19158		**Exon 17**	
382	**SCN4A probe** 16629-L19159			**Exon 15**
391 *	Reference probe 06672-L27891	10p15		
408	**CACNA1S probe** 17931-L22237		**Exon 24**	
427	**CACNA1S probe** 17932-L22854		**Exon 29**	
436	**CACNA1S probe** 16633-L19163		**Exon 38**	
445	**SCN4A probe** 16634-L19164			**Exon 3**
452 *	Reference probe 19636-L26295	10p11		
463	**SCN4A probe** 16635-L19165			**Exon 2**
472	**CACNA1S probe** 16636-L19166		**Exon 44**	
490	Reference probe 14883-L22098	14q11		

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

* New in version A3.
¥ Changed in version A3. Minor alteration, no change in sequence detected.

Table 2. P397-A3 probes arranged according to chromosomal location

Table 2a. *CACNA1S*

Length (nt)	SALSA MLPA probe	CACNA1S exon[a]	Ligation site NM_000069.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*88-90 (Exon 1)*		
238	16616-L19146	Exon 1	88 nt before exon 1	CTTGCTGGGAGC-GAGGAGAGTAAT	18.6 kb
172	21100-L29489	Exon 3	397-398	AAGCCGCCATGA-AGATCATTGCCT	2.3 kb
364	16627-L19157	Exon 5	769-770	AGACCTGCTACT-TCATTGGTACAG	6.2 kb
202	16611-L19141	Exon 8	1186-1187	TTCGGGGCTACA-TGAGCTGGATCA	8.5 kb
226	16614-L21583	Exon 12	1869-1870	GAAGTACGGCGC-AGCAACTTTGAC	6.6 kb
372	16628-L19158	Exon 17	2397-2398	AAAGAGAAGGCC-GTGCCCATTCCA	3.4 kb
184	16608-L19138	Exon 20	2734-2735	CCCTCATCTCCA-TGGGACTTGAGT	4.8 kb
408	17931-L22237	Exon 24	3012-3013	TACTACGTGTAC-AAGGACGGGGAC	7.6 kb
427	17932-L22854	Exon 29	3749-3750	AGGCTGCGGGAA-CGTTGTAATTTC	3.4 kb
208	16612-L19142	Exon 33	3 nt before exon 33	TCTTCTGTCCTC-TAGGTGTGCAAC	2.1 kb
296	16621-L21585	Exon 35	4369-4370	CCCTGCTGAGAA-GGATTCAGCCCC	1.9 kb
436	16633-L19163	Exon 38	4730-4731	TGGCTATCGGCC-CAAGAAGGACAT	7.2 kb
472	16636-L19166	Exon 44	5575-5576	AGATCATGGCAA-CAGAGCTACTGA	
		stop codon	*5707-5709 (Exon 44)*		

Table 2b. *SCN4A*

Length (nt)	SALSA MLPA probe	SCN4A exon[a]	Ligation site NM_000334.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*78-80 (Exon 1)*		
141	16603-L19133	Exon 1	80-81	GATGCGAGGATG-GCCAGACCATCT	0.4 kb
463	16635-L19165	Exon 2	365-366	TTCATCGTACTC-AACAAGGGCAAG	0.2 kb
445	16634-L19164	Exon 3	24 nt before exon 3	CTAGCTGCTTCC-TCACTTTCCTTG	0.5 kb
196	16610-L19140	Exon 4	626-627	GGCTTCTGTGTC-GACGACTTCACA	3.7 kb
256	16618-L19148	Exon 6	1026-1027	ACGGCAATGACT-CATGGTATGCCA	1.9 kb
136	16602-L19132	Exon 8	1215-1216	TCAAGACCGGGC-GGAACCCCAACT	1.6 kb
221	16613-L19143	Exon 9	1365-1366	TCTTCGTGGTCA-TCATCTTCCTGG	0.8 kb
234	16615-L22853	Exon 10	1541-1542	GCCAAGGCCGCC-CAAGCTCTGGAA	4.5 kb
166	16607-L19137	Exon 12	2064-2065	TGGCCAACGTAC-AGGGACTGTCTG	2.1 kb
148	16604-L19134	Exon 13	2447-2448	GTCATCGGCAAT-CTTGTGGTGAGT	5.7 kb
288	16622-L21584	Exon 14	2832-2833	TCAACAACCCCT-ACCTGACCATAC	2.0 kb
382	16629-L19159	Exon 15	2951-2952	CAGCCTCTCTAT-GATGGGAACTCG	0.8 kb
282	16620-L19150	Exon 16	3125-3126	CGTGGGAAGAAG-TGGTGGACTCTG	1.6 kb
160	16606-L19136	Exon 18	3425-3426	GTGGCCAACTGG-CTGGGCTACTCG	1.7 kb
335	16624-L19154	Exon 19	3685-3686	CATCTCCGAGGT-CAACAACAAGTC	0.5 kb
244	16617-L19147	Exon 20	23 nt after exon 20	TCGCTGAGATGT-GGCTGGTGAGCG	0.2 kb
319	16623-L19153	Exon 21	3866-3867	GAGGAGCAGCCG-CAGTACGAGGTG	1.8 kb
274	16619-L19149	Exon 23	4234-4235	GGACATCCTGTA-CAACATCAACAT	1.4 kb
355	17933-L22239	Exon 24	4794-4795	GCAACCCCTCCA-TCGGCATCTGCT	
		stop codon	*5586-5588 (Exon 24)*		

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.

Related SALSA MLPA probemixes

P137 SCN1A	Contains probes for all 26 coding exons of the *SCN1A* gene.
P279 CACNA1A	Contains probes for the *CACNA1A* gene.
P348 ATP1A2-CACNA1A	Contains 17 more probes for the *CACNA1A* gene, as well as probes for most *ATP1A2* exons. All *CACNA1A* probes in the P348 are different compared to the P279 probes.
P350 CLCN1-KCNJ2	Contains probes for the *CLCN1* gene, involved in myotonia congenita.

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.

P397 Product history	
Version	*Modification*
A3	One reference probe has been removed, three reference probes have been replaced, and one reference probe length has been adjusted.
A2	One reference probe has been replaced and one removed. In addition the length of one probe has been adjusted.
A1	First release.

Implemented changes in the product description

Version A3-01 – 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 *CACNA1S* gene updated according to new version of the NM_ reference sequence.

Version 05 – 15 August 2016 (55)
- Product description adapted to a new version (lot number changed, small changes in Table 1 and 2, new picture included).
- Various minor textual changes throughout the document.

Version 04 – 03 March 2016 (55)
- New related probemix added.
- Manufacturer's address adjusted.

Version 03 – 30 June 2015 (54)
- Electropherogram picture of the old buffer (introduced Dec. 2012) removed.

Version 02 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 01 (48)
- New document. Not applicable.

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