Product Description SALSA® MLPA® Probemix P108 SCN5A

To be used with the MLPA General Protocol.

Version B4. As compared to version B3, three reference probes have been replaced. For complete product history see page 6.

Catalogue numbers:
- **P108-025R:** SALSA MLPA Probemix P108 SCN5A, 25 reactions.
- **P108-050R:** SALSA MLPA Probemix P108 SCN5A, 50 reactions.
- **P108-100R:** SALSA MLPA Probemix P108 SCN5A, 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 P108 SCN5A is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SCN5A* gene, which is associated with Brugada syndrome-1 and long QT syndrome type 3.

The Brugada syndrome-1 and long QT syndrome type 3 are two hereditary cardiac diseases associated with mutations in the *SCN5A* gene. The SCN5A protein is found primarily in cardiac muscle and mediates the voltage-dependent sodium ion permeability of excitable membranes. Both syndromes are ion channel diseases of the heart. Brugada syndrome-1 is characterised by ST-segment elevation on surface electrocardiogram and long QT syndrome type 3 by a prolonged QT interval. Both disorders can lead to sudden death, which may be the first manifestation of the disease. Apart from these syndromes, mutations in the *SCN5A* gene are associated with several other cardiomyopathies such as familial atrial fibrillation, dilated cardiomyopathy, Romano-Ward syndrome and sick sinus syndrome 1.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1517/ and https://www.ncbi.nlm.nih.gov/books/NBK1129/.

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 *SCN5A* exon numbering used in this P108-B4 SCN5A product description is the exon numbering from the RefSeq transcript NM_198056.2, which is identical to the LRG_289 sequence. The exon numbering and NM_ sequence used have been retrieved in 07/2019. 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 P108-B4 SCN5A contains 40 MLPA probes with amplification products between 142 and 453 nucleotides (nt). This includes 31 probes for the *SCN5A* gene.

In addition, nine 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 Brugada syndrome-1 and long QT syndrome type 3. 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 *SCN5A* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P108 SCN5A.
- 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.

***SCN5A* mutation database:** https://databases.lovd.nl/shared/genes/SCN5A 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 *SCN5A* exons 3 and 5 but not exon 4) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P108-B4 SCN5A

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SCN5A*
64-105	Control fragments – see table in probemix content section for more information		
142	Reference probe 14199-L29536	2q12	
148	**SCN5A probe** 03564-L02930		**Exon 16**
154 *	Reference probe 16315-L20696	7p15	
160	**SCN5A probe** 03557-L09329		**Exon 2**
166	**SCN5A probe** 15391-L17222		**Exon 18**
172	Reference probe 10143-L10605	18q11	
178	**SCN5A probe** 03558-L02924		**Exon 3**
184	**SCN5A probe** 03566-L02932		**Exon 20**
190 #	**SCN5A probe** 07815-L07545		**Exon 21**
202	**SCN5A probe** 03559-L02925		**Exon 4**
209	**SCN5A probe** 15390-L17787		**Exon 19a**
214	**SCN5A probe** 03567-L09330		**Exon 22**
222	Reference probe 05709-L15344	3q21	
229	**SCN5A probe** 15386-L17217		**Exon 7**
241	**SCN5A probe** 03568-L29551		**Exon 23**
247	**SCN5A probe** 07812-L09326		**Exon 15**
256	**SCN5A probe** 03561-L02927		**Exon 8**
265	**SCN5A probe** 03569-L02935		**Exon 24**
274 *	Reference probe 08887-L08849	12q21	
284	**SCN5A probe** 03562-L02928		**Exon 10**
292	**SCN5A probe** 03570-L02936		**Exon 29**
298	**SCN5A probe** 15388-L17790		**Exon 5**
305 #	**SCN5A probe** 15387-L17789		**Exon 29**
312	**SCN5A probe** 15380-L17791		**Exon 12**
319	**SCN5A probe** 15385-L17792		**Exon 28**
328	Reference probe 19605-L29238	8q13	
337 «	**SCN5A probe** 03579-L02940		**Exon 1**
346	**SCN5A probe** 03580-L02941		**Exon 26**
355	**SCN5A probe** 07813-L07543		**Exon 17**
364 *	Reference probe 11592-L12339	16q21	
373	**SCN5A probe** 15382-L17213		**Exon 25**
382	**SCN5A probe** 07808-L07538		**Exon 9**
391	**SCN5A probe** 15395-L17793		**Exon 6**
400	Reference probe 14204-L15818	11p15	
409	**SCN5A probe** 15392-L17223		**Exon 13**
418	**SCN5A probe** 15394-L17225		**Exon 11**
427	**SCN5A probe** 07817-L07547		**Exon 27**
436	**SCN5A probe** 15389-L17220		**Exon 14**
445 «	**SCN5A probe** 15383-L17214		**Upstream**
453	Reference probe 11712-L12483	17q25	

a) See above section on exon numbering for more information.
* New in version B4.
« 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.
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.

Table 2. *SCN5A* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*SCN5A* exon[a]	Ligation site NM_198056.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
445 «	15383-L17214	Upstream	1746 nt before exon 1	GAAACGTTGACA-CTGCCTGCACTT	2.1 kb
337 «	03579-L02940	Exon 1	232 nt after exon 1	CGTCTCTAAACA-CCGTGCGCCCCC	16.0 kb
		start codon	*195-197 (exon 2)*		
160	03557-L09329	Exon 2	240-241	GCTTCCGCAGGT-TCACACGGGAGT	2.9 kb
178	03558-L02924	Exon 3	528-529	CCAACGCCTTGT-ATGTCCTCAGTC	7.9 kb
202	03559-L02925	Exon 4	633-634	CCAACTGCGTGT-TCATGGCCCAGC	1.6 kb
298	15388-L17790	Exon 5	794-793 reverse	GCCATGATAATC-ACACTAAAGTCC	6.9 kb
391	15395-L17793	Exon 6	890-891 in NM_001099404.1	ACTATTTCAGTT-ATCCCAGGTAAG	0.1 kb
229	15386-L17217	Exon 7	811-810 reverse	CAAATTCAGTTG-TGTATCTGTAAC	4.0 kb
256	03561-L02927	Exon 8	985-986	CCTCAGCGTCTT-TGCCCTCATCGG	1.7 kb
382	07808-L07538	Exon 9	1165-1166	CTCTGATGTGTT-ACTGTGTGGGAA	1.4 kb
284	03562-L02928	Exon 10	1248-1249	CCGACCACGGCT-ACACCAGCTTCG	0.7 kb
418	15394-L17225	Exon 11	1466-1467	CAAGCCACCATC-GCTGAGACCGAG	1.3 kb
312	15380-L17791	Exon 12	1706-1707	GATGGTCCCAGA-GCAATGGTAATC	0.9 kb
409	15392-L17223	Exon 13	1967-1968	CATGGCAAAAAG-AACAGCACTGTG	4.9 kb
436	15389-L17220	Exon 14	2201-2202	GTCAGCGTCCTC-ACCAGCGCACTG	1.1 kb
247	07812-L09326	Exon 15	2348-2349	ATGGACCCGTTT-ACTGACCTCACC	10.4 kb
148	03564-L02930	Exon 16	2596-2597	CCTGTCCCGCAT-GAGCAACTTGTC	1.7 kb
355	07813-L07543	Exon 17	2961-2962	TCTTGCTTGTTA-TGGTCATTGGCA	4.6 kb
166	15391-L17222	Exon 18	3262-3261 reverse	GTGTTTCCTTGC-GGGTGGGAGGCA	1.7 kb
209	15390-L17787	Exon 19a	3531-3532	GTGCATCTCAGG-CCGACTGGCGGC	2.7 kb
184	03566-L02932	Exon 20	3640-3641	CAACACCGCTGA-GCTCCTGGAGCA	1.4 kb
190 #	07815-L07545	Exon 21	3821-3822	TGGTTCGAGACA-TTCATCATCTTC	8.8 kb
214	03567-L09330	Exon 22	3936-3937	AGATGTTCACAT-ATGTCTTCGTGC	4.0 kb
241	03568-L29551	Exon 23	4108-4109	ACTGCGGACGCT-GCGTGCACTCCG	2.2 kb
265	03569-L02935	Exon 24	4344-4345	ACAAGAGCCAGT-GTGAGTCCTTGA	2.9 kb
373	15382-L17213	Exon 25	46 nt before exon 25	GAAGCTCAAGCG-AGGTACAGAATT	0.8 kb
346	03580-L02941	Exon 26	4550-4549 reverse	AAGATGATGAAA-ATGACAAAATAG	0.8 kb
427	07817-L07547	Exon 27	5 nt before exon 27	GTGCCTTCTCTT-TGCACTTAGGGG	1.3 kb
319	15385-L17792	Exon 28	4800-4799 reverse	GCAGATCAGAAA-CATGATGGTGAC	3.0 kb
292	03570-L02936	Exon 29	5035-5036	CATCATCCAGAA-GTACTTCTTCTC	0.8 kb
305 #	15387-L17789	Exon 29	5846-5845 reverse	GAGATCTTGGAT-GGGTTGGCTGCC	
		stop codon	*6243-6245 (exon 29)*		

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.

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.

Related SALSA MLPA probemixes

P114 Long-QT — Contains probes for the *KCNQ1*, *KCNH2*, *KCNE1* and *KCNE2* genes, which are involved in congenital long QT syndrome.

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 P108 SCN5A

- Broendberg et al. (2016). Repeated molecular genetic analysis in Brugada syndrome revealed a novel disease-associated large deletion in the *SCN5A* gene. *Heart Rhythm Case Rep.* 2(3):261-4.
- Broendberg et al. (2018). Targeted next generation sequencing in a young population with suspected inherited malignant cardiac arrhythmias. *Eur J Hum Genet*. 26:303-13.
- Garcia-Molina et al. (2013). A study of the *SCN5A* gene in a cohort of the 76 patients with Brugada syndrome. *Clin Genetics.* 83(6): 530-8.
- Eastaugh et al. (2011). Brugada syndrome caused by a large deletion in *SCN5A* only detected by multiplex ligation-dependent probe amplification. *J Cardiovasc Electrophysiol*. 22:1073-6.
- Macarie et al. (2009). The electrocardiographic abnormalities in highly trained athletes compared to the genetic study related to causes of unexpected sudden cardiac death. *J Med Life*. 2:361-72.
- Medemont-Soler et al. (2016). Large genomic imbalances in Brugada syndrome. *PLoS ONE.* 11(9):e0163514.
- Selga et al. (2015). Comprehensive genetic characterization of a Spanish Brugada syndrome cohort. *PLos ONE.* 10(7):e0132888.
- Sonoda et al. (2018). Copy number variations of *SCN5A* in Brugada syndrome. *Heart Rhythm*. 15(8):1179-88.

P108 Product history	
Version	*Modification*
B4	Three reference probes have been replaced.
B3	Two reference probes have been replaced.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	Nine SCN5A probes and several reference probes have been replaced. The number of SCN5A probes has been increased to 31.
A	First release.

Implemented changes in the product description

Version B4-01 — 10 September 2019 (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).
- Various minor textual or layout changes.

Version 13 – 11 October 2016 (55)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Reference added at page 1.
- Several minor textual changes throughout the document.

Version 12 – 23 July 2015 (54)
- Figure based on the use of old MLPA buffer (replaced in December 2012) removed.
- Various minor textual changes throughout the document.

More information: www.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mlpa.com (information & technical questions); order@mlpa.com (orders)
Phone	+31 888 657 200