Product Description SALSA® MLPA® Probemix P279-C1 CACNA1A

To be used with the MLPA General Protocol.

Version C1. As compared to version B1, three target probes have been added, one target and six reference probes have been replaced. In addition, two reference probes have been removed and four probe lengths have been adjusted. For complete product history see page 7.

Catalogue numbers:
- **P279-025R:** SALSA MLPA Probemix P279 CACNA1A, 25 reactions.
- **P279-050R:** SALSA MLPA Probemix P279 CACNA1A, 50 reactions.
- **P279-100R:** SALSA MLPA Probemix P279 CACNA1A, 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 P279 CACNA1A is a **research use only (RUO)** assay for the detection of deletions or duplications in the genes *CACNA1A*, which is associated with familial hemiplegic migraine and episodic ataxia 2, and *KCNA1*, which is involved in episodic ataxia 1.

Familial hemiplegic migraine (FHM) falls within the category of migraine with aura and is characterised by hemiparesis in combination with at least one symptom of aura, such as visual disturbance, sensory loss, and dysphasia. Neurologic deficits with FHM attacks can be prolonged for hours to days and may outlast the associated migrainous headache. Approximately 7% of FHM cases are attributed to pathogenic variants of *CACNA1A*, located on chromosome 19p13.13. Other genes, including *SCN1A*, *ATP1A2* and *PRRT2* have also been associated to FHM. These genes are covered by probemixes P137 SCN1A and P348 ATP1A2-CACNA1A-PRRT2.

Episodic ataxia type 2 (EA2) involves episodes of ataxia including vertigo and nausea. Attacks can also be associated with dysarthria, diplopia, tinnitus, dystonia, hemiplegia and headache, and typically last minutes to days. EA2 is caused by pathogenic variants of *CACNA1A* and is inherited in an autosomal dominant manner. Overlap has been described between the FHM phenotype associated with pathogenic missense variants of *CACNA1A* and EA2.

Episodic ataxia type 1 (EA1) is characterized by constant myokymia and episodes of spastic contractions of the skeletal muscles of the head, arms, and legs with loss of both motor coordination and balance. During attacks individuals may experience a number of variable symptoms including vertigo, blurred vision, diplopia, nausea, headache, diaphoresis, clumsiness, stiffening of the body, dysarthric speech, and difficulty in breathing. EA1 is caused by pathogenic variants of *KCNA1*, located on chromosome 12p13.32, and is inherited in an autosomal dominant manner.

More information is available for
Familial hemiplegic migraine: https://www.ncbi.nlm.nih.gov/books/NBK1501/
Episodic ataxia type 2: https://www.ncbi.nlm.nih.gov/books/NBK1388/
Episodic ataxia type 1: https://www.ncbi.nlm.nih.gov/books/NBK25442/

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 *CACNA1A* exon numbering used in this P279-C1 CACNA1A product description is the exon numbering from the RefSeq transcript NM_001127221.1, which is identical to the LRG_7 sequence. The *KNCA1* exon numbering used in this product description is the exon numbering from the RefSeq transcript NM_000217.3, which is identical to the LRG_1297 sequence. The exon numbering and NM_ sequences used have been retrieved on 08/2020. 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 P279-C1 CACNA1A contains 46 MLPA probes with amplification products between 130 and 487 nucleotides (nt). This includes 34 probes for the *CACNA1A* gene, one flanking probe targeting the *LDLR* gene downstream of *CACNA1A*, and two probes for the *KCNA1* 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 FHM, EA2 and EA1. 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 or in or near the *CACNA1A* gene. 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 *CACNA1A* and *KCNA1* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P279 CACNA1A.
- 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.

Mutation databases:
CACNA1A: https://databases.lovd.nl/shared/genes/CACNA1A.
KCNA1: https://databases.lovd.nl/shared/genes/KCNA1.
We strongly encourage users to deposit positive results in 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 *CACNA1A* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P279-C1 CACNA1A

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	CACNA1A	KCNA1
64-105	Control fragments – see table in probemix content section for more information			
130 *	Reference probe 10499-L11052	7q34		
136	**CACNA1A probe** 09067-L09236		**Exon 16**	
142	**CACNA1A probe** 13532-L29524		**Exon 19**	
148 «	**CACNA1A probe** 09082-L29525		**Exon 43**	
154	Reference probe 19447-L25861	14q31		
160	**CACNA1A probe** 09074-L09243		**Exon 27**	
166	**CACNA1A probe** 09079-L09248		**Exon 38**	
172	**CACNA1A probe** 13533-L14993		**Exon 5**	
178 * «	**CACNA1A probe** 22822-L32184		**Exon 41**	
184	**CACNA1A probe** 09062-L09231		**Exon 7**	
191 *	Reference probe 12749-L30862	17p13		
196 ¥	**CACNA1A probe** 09076-L32323		**Exon 31**	
202	**CACNA1A probe** 13534-L14994		**Exon 2**	
208	**KCNA1 probe** 11611-L12371			**Exon 1**
215	**CACNA1A probe** 09066-L23736		**Exon 13**	
226 «	**CACNA1A probe** 09080-L09249		**Exon 39**	
232	**CACNA1A probe** 09063-L09232		**Exon 8**	
241	**CACNA1A probe** 09072-L23737		**Exon 24**	
250 «	**CACNA1A probe** 09081-L09250		**Exon 40**	
257	**CACNA1A probe** 09064-L16631		**Exon 10**	
265 «	**CACNA1A probe** 13535-L14995		**Exon 42**	
273	**CACNA1A probe** 09075-L09244		**Exon 29**	
280 *	Reference probe 13350-L26120	9q21		
288 * «	**CACNA1A probe** 22824-L32367		**Exon 46**	
301	**CACNA1A probe** 21111-L29526		**Exon 1**	

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	CACNA1A	KCNA1
310	**CACNA1A probe** 09065-L09234		**Exon 11**	
319	Reference probe 01017-L00322	18q12		
328	**CACNA1A probe** 21112-L29527		**Exon 6**	
342 ¥	**CACNA1A probe** 09073-L24818		**Exon 25**	
348 ¥	**CACNA1A probe** 09078-L32324		**Exon 35**	
355	**KCNA1 probe** 11614-L12374			**Exon 2**
364 *	**CACNA1A probe** 22826-L32187		**Exon 4**	
373 *	Reference probe 18296-L25750	8p12		
382	**CACNA1A probe** 09068-L09237		**Exon 18**	
391	**CACNA1A probe** 09056-L09225		**Exon 1**	
400 ¬	**LDLR probe** 03004-L02443		**Downstream**	
409	**CACNA1A probe** 13537-L14997		**Exon 3**	
415	**CACNA1A probe** 09070-L09239		**Exon 20**	
422 * «	**CACNA1A probe** 22827-L32298		**Exon 45**	
430 *	Reference probe 02990-L09995	16p13		
436	**CACNA1A probe** 09077-L09246		**Exon 33**	
445 «	**CACNA1A probe** 09084-L09253		**Exon 47**	
452 *	Reference probe 19175-L25143	13q22		
467 ¥	**CACNA1A probe** 13538-L32325		**Exon 32**	
476	**CACNA1A probe** 13539-L14999		**Exon 22**	
487	Reference probe 09870-L10627	2p15		

* New in version C1.
¥ Changed in version C1. 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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

Table 2. P279-C1 probes arranged according to chromosomal location

Table 2a. *CACNA1A* gene

Length (nt)	SALSA MLPA probe	CACNA1A exon[a]	Ligation site NM_001127221.1	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*237-239 (Exon 1)*		
301	21111-L29526	Exon 1	385-386	GATGTACAAGCA-GTCAATGGCGCA	0.1 kb
391	09056-L09225	Exon 1	503-504	GTGGTGAGAAAA-TACGCCAAAAAG	50.9 kb
202	13534-L14994	Exon 2	13 nt after exon 2	TGAGTGATGTCT-TTTCTCAGGGTC	2.1 kb
409	13537-L14997	Exon 3	662-663	TACTTCATTGGA-ATTTTTTGTTTC	81.3 kb
364	22826-L32187	Exon 4	845-846	CTGCGGCCGCTC-AAGCTGGTGTCT	6.3 kb
172	13533-L14993	Exon 5	890-891	GTCCTGAAGTCG-ATCATGAAGGCG	5.8 kb
328	21112-L29527	Exon 6	1169-1170	GTGCTGACTGTT-TTCCAGTGCATA	23.7 kb
184	09062-L09231	Exon 7	1215-1216	ACACCCTACAGA-GCAACGATGCCT	1.5 kb
232	09063-L09232	Exon 8	1390-1391	GCAACAACAGAT-TGAACGTGAGCT	4.1 kb
257	09064-L16631	Exon 10	1519-1520	CACCATAAAGAA-AAGCAAGACAGA	13.1 kb
310	09065-L09234	Exon 11	1704-1705	CTCAGGCCTTCT-ACTGGACTGTAC	8.7 kb
215	09066-L23736	Exon 13	1950-1951	GGGCTGTCATAA-AACCTGGCACAT	4.6 kb
136	09067-L09236	Exon 16	2257-2258	GAACGAGGTCAT-GTACGACGGGAT	3.2 kb
382	09068-L09237	Exon 18	2460-2461	TTGCCCTACAGA-AAGCCAAGGAGG	1.3 kb
142	13532-L29524	Exon 19	2580-2581	GGACCAGTGAGA-TGCGAAAGCAGA	12.4 kb
415	09070-L09239	Exon 20	3447-3448	ACAACATGAAGA-ACAACAAGCTGG	3.5 kb
476	13539-L14999	Exon 22	3957-3958	GCCATTACATCC-TGAACCTGCGCT	7.5 kb
241	09072-L23737	Exon 24	4180-4181	CCTCTGGAATAT-TCTCGACTTCAT	13.1 kb
342	09073-L24818	Exon 25	4254-4255	GAAAAGACATCA-ACACGATTAAAT	3.2 kb
160	09074-L09243	Exon 27	4557-4558	AGAAGTATGAAT-TCCATTACGACA	4.5 kb
273	09075-L09244	Exon 29	4944-4945	CGCCTTTCGAGT-ACACGATCATGG	9.9 kb
196	09076-L32323	Exon 31	5111-5112	TTCTAGAATTAT-TTCCGCGATGCC	9.6 kb

467	13538-L32325	Exon 32	5302-5303	TGTGCAGTCCTT-CAAGGTGAGTCC	0.4 kb
436	09077-L09246	Exon 33	5341-5342	GATCGCCATGCT-CTTCTTCATCTA	3.4 kb
348	09078-L32324	Exon 35	5518-5519	TTGGCACAACAT-CATGCTTTCCTG	7.1 kb
166	09079-L09248	Exon 38	5918-5919	GTCCACTTCAAT-TCCACCCTCATG	10.2 kb
226 «	09080-L09249	Exon 39	6026-6027	ATGATGGCGATT-TGGCCCAATCTG	0.3 kb
250 «	09081-L09250	Exon 40	6124-6125	CATGATGATCAT-GGAGTACTACCG	1.6 kb
178 «	22822-L32184	Exon 41	6208-6209	GTTCCAGCGCAT-GGAGCCCCCGTC	0.2 kb
265 «	13535-L14995	Exon 42	25 nt before exon 42	GCGCCCACTGCT-ACCCCGGCCTCT	0.4 kb
148 «	09082-L29525	Exon 43	6458-6459	ATGGGCAGAGAT-GGCTACTCCGAC	2.7 kb
422 «	22827-L32298	Exon 45	6630-6631	TGCTGGGCCCCA-AGGCCCGACGCC	0.7 kb
288 «	22824-L32367	Exon 46	6993-6994	CGCCCAGCGAGG-GCCGAGAGCACA	0.7 kb
445 «	09084-L09253	Exon 47	7021-7022	TGTAGGGCAGTA-GTTCCGTAAGTG	2088.0 kb
		stop codon	*7020-7022 (Exon 47)*		
400 ¬	03004-L02443	LDLR gene		TCAGTGCCAACC-GCCTCACAGGTT	

« 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Table 2b. *KNCA1* gene

Length (nt)	SALSA MLPA probe	KCNA1 exon[a]	Ligation site NM_000217.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*1108-1110 (Exon 2)*		
208	11611-L12371	Exon 1	217-218	GAGAGTGCTGTT-TATCGTCATTTG	4.4 kb
355	11614-L12374	Exon 2	4227-4228	AACTAAACCAAT-TGATTTAATAGT	
		stop codon	*2593-2595 (Exon 2)*		

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 the gene *SCN1A*, which is related seizure disorders including Dravet syndrome and Familial hemiplegic migraine 3.

P348 ATP1A2-CACNA1A-PRRT2 — Contains probes for the genes *ATP1A2*, *CACNA1A* and *PRRT2*, which are associated to familial hemiplegic migraine. The 17 *CACNA1A* probes in P348 are different from the probes in P279.

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 P279 CACNA1A

- Labrum RW et al. (2009). Large scale calcium channel gene rearrangements in episodic ataxia and hemiplegic migraine: implications for diagnostic testing. *J Med Genet*. 46:786-91.
- Sintas C et al. (2017). Mutation spectrum in the CACNA1A gene in 49 patients with episodic ataxia. *Sci Rep*. 7:2514.
- Wan J et al. (2011). Large genomic deletions in *CACNA1A* cause episodic ataxia type 2. *Front Neurol*. 2:51.

P279 Product history	
Version	*Modification*
C1	Three target probes have been added, one target and six reference probes have been replaced. In addition, two reference probes have been removed and four probe lengths have been adjusted.
B3	One reference probe has been replaced and the length of several probes has been adjusted.
B2	One reference probe has been replaced and three reference probes have been removed. In addition, the control fragments have been adjusted (QDX2).
B1	Additional probes for *CACNA1A* and *KCNA1* have been added and several probes have been adjusted.
A2	X and Y chromosome specific control fragments at 100 and 105 nt have been added.
A1	First release.

Implemented changes in the product description

Version C1-01 — 28 August 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 *KCNA1* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 14 – 28 September 2016 (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).
- Various minor textual changes on pages 1 and 2.
- New references added.
- Exon numbering of the *CACNA1A* gene has been changed the Table 1 and Table 2a.

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