Product Description SALSA® MLPA® Probemix P348-C1 ATP1A2-CACNA1A-PRRT2

To be used with the MLPA General Protocol.

Version C1. As compared to version B1, probes for *PRRT2* have been added, four reference probes have been replaced, one reference probe has been removed, and six probes have been adjusted in length. For complete product history see page 8.

Catalogue numbers:
- **P348-025R:** SALSA MLPA Probemix P348 ATP1A2-CACNA1A-PRRT2, 25 reactions.
- **P348-050R:** SALSA MLPA Probemix P348 ATP1A2-CACNA1A-PRRT2, 50 reactions.
- **P348-100R:** SALSA MLPA Probemix P348 ATP1A2-CACNA1A-PRRT2, 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.mrcholland.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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 P438 ATP1A2-CACNA1A-PRRT2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *ATP1A2*, *CACNA1A* and *PRRT2* genes, which are associated with familial hemiplegic migraine (FHM).

Migraine is a common neurological disorder that affects up to 15% of the general population (Vos et al. 2012). FHM is a rare autosomal dominantly inherited subtype of migraine with aura. In case of FHM, the aura usually consist of a phase with hemiparesis accompanied by typical aura symptoms, including visual, sensory or speech disturbances, followed by a headache phase (Jen JC. 2015). Four genes have been identified for different types of FHM: *CACNA1A*, *ATP1A2*, *SCN1A* and the more recently identified gene *PRRT2* (Friedrich et al. 2016).

The *ATP1A2* gene (23 exons) spans ~28 kb of genomic DNA and is located on 1q23.2, ~160 Mb from the p-telomere. The *CACNA1A* gene (47 exons) spans ~300 kb of genomic DNA and is located on 19p13, ~13 Mb from the p-telomere. The *PRRT2* gene (4 exons) spans ~3.7 kb of genomic DNA and is located on 16p11.2, ~30 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1388/.

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 *ATP1A2*, *CACNA1A* and *PRRT2* exon numbering used in this P348-C1 ATP1A2-CACNA1A-PRRT2 product description is the exon numbering from the LRG_6, LRG_7 and NG_032039.1 sequences, respectively. The exon numbering used has been retrieved on 04/2020. 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 P348-C1 ATP1A2-CACNA1A-PRRT2 contains 49 MLPA probes with amplification products between 130 and 511 nucleotides (nt). This includes 21 probes for *ATP1A2*, 15 probes for *CACNA1A* and five probes for *PRRT2*. In addition, eight 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.mrcholland.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.mrcholland.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.mrcholland.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 familial hemiplegic migraine. 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. Sample ID numbers NA05875 (heterozygous *PRRT2* deletion) and NA00803 (heterozygous *ATP1A2* deletion) from the Coriell Institute have been tested with this P348-C1 probemix at MRC Holland and can be used as positive control samples to detect copy number alterations. 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.mrcholland.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 *PRRT2* 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 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 *ATP1A2*, *CACNA1A* and *PRRT2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P348 ATP1A2-CACNA1A-PRRT2.
- 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.

***ATP1A2*/*CACNA1A*/*PRRT2* mutation databases:** https://databases.lovd.nl/shared/genes/ATP1A2, https://databases.lovd.nl/shared/genes/CACNA1A, https://databases.lovd.nl/shared/genes/PRRT2. 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 *ATP1A2* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P348-C1 ATP1A2-CACNA1A-PRRT2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*ATP1A2*	*CACNA1A*	*PRRT2*
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 08640-L08656	3q26			
136 * «	**PRRT2 probe** 22454-L31630				**Exon 1**
142 ¥	**ATP1A2 probe** 13556-L31634		**Exon 9**		
148	**ATP1A2 probe** 21109-L29671		**Exon 3**		
154	**CACNA1A probe** 13560-L15872			**Exon 15**	
160	**ATP1A2 probe** 13559-L15867		**Exon 17**		
166	**ATP1A2 probe** 13561-L15018		**Exon 11**		
175	Reference probe 10416-L12862	9q34			
182	**CACNA1A probe** 13562-L23172			**Exon 30**	
188	**ATP1A2 probe** 13563-L23173		**Exon 10**		
196 ¥	**ATP1A2 probe** 22465-L32062		**Exon 16**		
202	**CACNA1A probe** 13566-L15023			**Exon 36**	
208 Ж	**ATP1A2 probe** 13567-SP0122-L15024		**Exon 2**		
214 * «	**PRRT2 probe** 22466-L32075				**Exon 2**
220	**ATP1A2 probe** 13568-L15025		**Exon 22**		
226 *	Reference probe 22098-L31085	7q22			
232	**ATP1A2 probe** 13570-L15027		**Exon 6**		
238	**CACNA1A probe** 13571-L15028			**Exon 26**	
244	**ATP1A2 probe** 13572-L15029		**Exon 14**		
250	**CACNA1A probe** 13573-L15030			**Exon 37**	
256 * «	**PRRT2 probe** 22467-L32064				**Exon 4**
262 Ж	**CACNA1A probe** 13574-SP0123-L15031			**Exon 9**	
268	**CACNA1A probe** 13575-L15032			**Exon 17**	
274 *	Reference probe 17921-L32065	14q23			
283	**CACNA1A probe** 13577-L23176			**Exon 14**	
288	**ATP1A2 probe** 13578-L23177		**Exon 20**		
296 ¥	**CACNA1A probe** 22468-L15026			**Exon 3**	
304 * «	**PRRT2 probe** 22469-L32056				**Exon 2**
317	**ATP1A2 probe** 13580-L15037		**Exon 12**		
328 Ж	**CACNA1A probe** 13581-SP0124-L15038			**Exon 28**	
335	**ATP1A2 probe** 21110-L29672		**Exon 8**		
346	**ATP1A2 probe** 13583-L15040		**Exon 5**		
355	**CACNA1A probe** 13584-L15041			**Exon 23**	
364	Reference probe 10088-L10512	8q22			
375	**ATP1A2 probe** 13585-L15042		**Exon 13**		
382 «	**CACNA1A probe** 13586-L15043			**Exon 44**	
392	**ATP1A2 probe** 13587-L15044		**Exon 15**		
400 ¥	**ATP1A2 probe** 13588-L24039		**Exon 4**		
409 Ж	**CACNA1A probe** 13589-SP0125-L15046			**Exon 21**	
418	**ATP1A2 probe** 13590-L23165		**Exon 21**		
427	Reference probe 14716-L16388	5q31			
441 ¥	**ATP1A2 probe** 13591-L32067		**Exon 1**		
454 * «	**PRRT2 probe** 22470-L31632				**Exon 3**
463	**ATP1A2 probe** 13593-L23166		**Exon 7**		
475	**CACNA1A probe** 13594-L15051			**Exon 34**	
481	**ATP1A2 probe** 13595-L15052		**Exon 19**		
490 *	Reference probe 19137-L25693	21q22			
504 ¥	**CACNA1A probe** 22464-L15871			**Exon 12**	
511 *	Reference probe 18539-L32066	17q11			

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

* 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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

Table 2. P348-C1 probes arranged according to chromosomal location

Table 2a. *ATP1A2* gene

Length (nt)	SALSA MLPA probe	*ATP1A2* exon[a]	Ligation site NM_000702.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*104-106 (Exon 1)*		
441	13591-L32067	Exon 1	17 nt after exon 1	TATCCCTAAAGA-GCAGGGGTCGCA	5.1 kb
208 Ж	13567-SP0122-L15024	Exon 2	190-191; 217-218	AAGGAGAAGGAA-27 nt spanning oligo-ATGGTGAGGGAA	0.3 kb
148	21109-L29671	Exon 3	265-266	CGCAAATACCAA-GTGGACCTGTCC	2.1 kb
400 #	13588-L24039	Exon 4	379-380	AAGTTCTGCCGT-CAGCTTTTCGGG	0.7 kb
346 #	13583-L15040	Exon 5	574-575	AAGATCATGGAT-TCCTTCAAGAAC	0.4 kb
232 #	13570-L15027	Exon 6	691-692	GGTGGAGACCGC-GTCCCTGCTGAC	0.8 kb
463	13593-L23166	Exon 7	751-752	AACTCATCCTTA-ACAGGAGAGTCG	2.4 kb
335	21110-L29672	Exon 8	880-881	GTGATTGCCACA-GGAGACCGGACG	1.1 kb
142	13556-L31634	Exon 9	1128-1129	CCAGGTGTGCCT-GACCCTGACAGC	0.3 kb
188	13563-L23173	Exon 10	1337-1338	CTTTTGACAAAC-GATCCCCTACGT	0.3 kb
166	13561-L15018	Exon 11	1502-1503	GCTCAGTGAGGA-AAATGAGAGACA	0.9 kb
317	13580-L15037	Exon 12	1684-1685	GAGATCCCGCTC-GACAAGGAGATG	0.3 kb
375	13585-L15042	Exon 13	1837-1838	TTTCCCACGGAG-AAGCTTTGCTTT	4.0 kb
244 #	13572-L15029	Exon 14	1996-1997	GTGGGCATCATA-TCAGAGGGTAAC	0.7 kb
392	13587-L15044	Exon 15	2152-2153	AAGAACCACACA-GAGATCGTCTTT	0.3 kb
196 #	22465-L32062	Exon 16	2266-2267	GACTCCCCTGCA-TTGAAGAAGGCT	0.5 kb
160	13559-L15867	Exon 17	2536-2537	GACCTGGGCACA-GATATGGTGAGC	0.7 kb
	No probe	Exon 18			
481	13595-L15052	Exon 19	2807-2808	GCTATGGACAGG-AGTGGGTGAGTG	0.3 kb
288	13578-L23177	Exon 20	2 nt after exon 20	AGGGCATGAAGT-GAGTGCCCACCC	2.7 kb
418	13590-L23165	Exon 21	4 nt after exon 21	CCGCTCAAGTGA-GTGTCTCTTTCG	0.1 kb
220	13568-L15025	Exon 22	3046-3047	ATTGCTTTCAGA-GTCACCTGGTGG	
	No probe	Exon 23			
		stop codon	*3164-3166 (Exon 23)*		

Table 2b. *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)*		
296	22468-L15026	Exon 3	732-733	GCTCCTACTTGA-GGAATGGCTGGA	120.0 kb
262 Ж	13574-SP0123-L15031	Exon 9	1463-1464; 2 nt after exon 9	GAGGATGAAACT-30 nt spanning oligo-AACTGCTCTAAA	20.2 kb
504	22464-L15871	Exon 12	19 nt after exon 12	CTCTTGTTTCTA-AGAGTTCATTTC	4.5 kb
283	13577-L23176	Exon 14	2138-2139	TTGGGAATGCAA-CTCTTCGGCGGC	0.3 kb
154	13560-L15872	Exon 15	2159-2160	TGCAGGTTTAAT-TTCGATGAAGGG	4.3 kb
268	13575-L15032	Exon 17	2405-2406	GCCCAGGAGCTC-ACCAAGGTGGAG	18.3 kb
409 Ж	13589-SP0125-L15046	Exon 21	50 nt before exon 21; 20 nt before exon 21	GAGTTCCCAGAG-30 nt spanning oligo-GTTGGTTCTTGT	8.1 kb
355	13584-L15041	Exon 23	4064-4065	CCCTTACAGGTG-CTGCGATACTTT	15.6 kb
238	13571-L15028	Exon 26	4394-4395	GTCTACATGCTA-TTCATGTTCATC	4.2 kb
328 Ж	13581-SP0124-L15038	Exon 28	4810-4811; 8 nt after exon 28	GATGGAGGAATA-27 nt spanning oligo-TCCAATTCCATC	4.3 kb
182	13562-L23172	Exon 30	5105-intron 30	TTTGGGATTCTG-GTAAGTACCACC	17.9 kb
475	13594-L15051	Exon 34	33 nt before exon 34	ACTGGAGGAATG-GCAGCCCCTGGT	4.9 kb
202	13566-L15023	Exon 36	5657-5658	AATCTCTTTGTC-GCCGTCATCATG	1.5 kb
250	13573-L15030	Exon 37	5834-5835	CTCGGCTTAGGC-AAGAAATGTCCT	18.1 kb
382 «	13586-L15043	Exon 44	6548-6549	TGACAGAGGAGA-AGGGGCCGGCCA	
		stop codon	*7020-7022 (Exon 47)*		

Table 2c. *PRRT2* gene

Length (nt)	SALSA MLPA probe	*PRRT2* exon[a]	Ligation site NM_145239.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*197-199 (Exon 2)*		
136 «	22454-L31630	Exon 1	127-128	GGAGGCCGGCGT-CGAGGTGAGACC	1.1 kb
214 «	22466-L32075	Exon 2	535-536	TCCAAACCAGAA-GTGAGCAAAGAG	0.4 kb
304 «	22469-L32056	Exon 2	919-920	TCTCCCCGAGGT-AGCCTGAGCCGC	0.6 kb
454 «	22470-L31632	Exon 3	1140-1141	GGTAGCCAAGCT-CTTAAGCATCGT	0.2 kb
256 «	22467-L32064	Exon 4	1218-1219	AGTGTATAAGTG-AGGGGCTCTGCC	
		stop codon	*1217-1219 (Exon 4)*		

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

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

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
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.
« 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

- P279 CACNA1A: Contains more probes for the *CACNA1A* gene.
- P137 SCN1A: Contains probes for the *SCN1A* gene.

References

- Friedrich T et al. (2016). ATP1A2 mutations in migraine: seeing through the facets of an ion pump onto the neurobiology of disease. *Front Physiol.* 7:239.
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- 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.
- Vos T et al. (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet.* 380(9859):2163-2196.

Selected publications using SALSA MLPA Probemix P348 ATP1A2-CACNA1A-PRRT2

- Gagliardi S et al. (2017). De novo exonic duplication of ATP1A2 in Italian patient with hemiplegic migraine: a case report. *J Headache Pain*. 18(1):63.
- Grieco GS et al. (2018). New CACNA1A deletions are associated to migraine phenotypes. *J Headache Pain.* 19(1):75

P348 Product history	
Version	*Modification*
C1	Probes for *PRRT2* have been added, four reference probes have been replaced, one reference probe has been removed, and six probes have been adjusted in length.
B1	Two target probes have been removed, two reference probes have been replaced and several probe lengths have been adjusted.
A3	QDX2 fragments have been added.
A2	Two reference probes have been removed.
A1	First release.

Implemented changes in the product description

Version C1-01 — 04 May 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 *ATP1A2* 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.
- Warning added to Table 2a for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Email addresses and website URLs updated.

Version 08 – 07 March 2017 (55)
- Warning added in Table 1 and Table 2b, 382 nt probe 13586-L15043.
- Various minor textual and layout changes.

Version 07 – 21 December 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 page 1.
- Exon numbering of the CACNA1A gene has been changed.

Version 06 (48)
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.

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