Product Description
SALSA® MLPA® Probemix P099-D1 GCH1-TH-SGCE-PRRT2

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 8.

Catalogue numbers
- **P099-025R:** SALSA® MLPA® Probemix P099 GCH1-TH-SGCE-PRRT2, 25 reactions
- **P099-050R:** SALSA® MLPA® Probemix P099 GCH1-TH-SGCE-PRRT2, 50 reactions
- **P099-100R:** SALSA® MLPA® Probemix P099 GCH1-TH-SGCE-PRRT2, 100 reactions

SALSA® MLPA® Probemix P099 GCH1-TH-SGCE-PRRT2 (hereafter: P099 GCH1-TH-SGCE-PRRT2) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P099-025R	**P099-050R**	**P099-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding 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
SALSA® MLPA® Probemix P099 GCH1-TH-SGCE-PRRT2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GCH1*, *TH*, *SGCE* and *PRRT2* genes, which are associated with autosomal

dominant dopa-responsive dystonia, autosomal recessive dopa-responsive dystonia, myoclonus-dystonia syndrome and paroxysmal movement disorders, respectively.

Autosomal dominant dopa-responsive dystonia (also known as autosomal dominant Segawa syndrome or GTP Cyclohydrolase 1-Deficient Dopa-Responsive Dystonia; OMIM #128230) is characterised by postural and motor disturbances showing marked diurnal fluctuation (Segawa et al. 1976). The disorder is caused by a mutation in the *GCH1* gene encoding GTP cyclohydrolase I. The GTP cyclohydrolase I enzyme is rate-limiting in the conversion of GTP to BH4, which is a cofactor for tyrosine hydroxylase (TH). TH is the rate-limiting enzyme for dopamine synthesis. The *GCH1* gene has 6 exons, spans ~61 kb of genomic DNA and is located on chromosome 14q22.2, ~55 Mb from the p-telomere. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1508/.

The autosomal recessive form of dopa-responsive dystonia (also known as autosomal recessive Segawa syndrome or Tyrosine hydroxylase deficiency; OMIM #605407) is commonly caused by deficiency of TH (De Lonlay et al. 2000). TH is involved in the conversion of tyrosine to dopamine. As the rate-limiting enzyme in the synthesis of catecholamines, tyrosine hydroxylase has a key role in the physiology of adrenergic neurons. The *TH* gene is located in the Beckwith-Wiedemann syndrome (BWS) critical region (Gu et al. 2002) on chromosome 11p15.5. The *TH* gene has 14 exons and spans ~8 kb of genomic DNA, ~2 Mb from the p-telomere. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1437/.

Myoclonus-dystonia syndrome (OMIM #159900) is caused by mutations in the *SGCE* gene encoding epsilon-sarcoglycan. The *SGCE* gene has 11 exons, spans ~71 kb of genomic DNA and is located on chromosome 7q21.3, ~94 Mb from the p-telomere. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1414/.

Several paroxysmal movement disorders have been associated with the *PRRT2* gene. In addition to these *PRRT2*-associated paroxysmal movement disorders, the gene has also been linked to other movement disorders and seizures. The *PRRT2* gene has 4 exons, spans ~4 kb of genomic DNA and is located on chromosome 16p11.2, ~29 MB from the p-telomere. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK475803/.

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

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/
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/

Exon numbering
The *GCH1*, *TH*, *SGCE* and *PRRT2* exon numbering used in this P099-D1 GCH1-TH-SGCE-PRRT2 product description is the exon numbering from the RefSeq transcript NM_000161.3, NM_199292.3, NM_003919.3 and NM_145239.3 sequences, respectively. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
P099-D1 GCH1-TH-SGCE-PRRT2 contains 39 MLPA probes with amplification products between 130 and 445 nucleotides (nt). This includes seven probes for GCH1, six probes for TH, thirteen probes for SGCE 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 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 using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families without a history of dopa-responsive dystonia (also known as Segawa disease) or other movement disorders. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. See the section of positive samples on the probemix product page on our website.

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 final ratio (FR) 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 FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of Coffalyser.Net. (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. Single Nucleotide Variants, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also 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: https://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.
- 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 warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, 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 *GCH1*, *TH*, *SGCE* and *PRRT2* genes are small (point) mutations, none of which will be detected by using P099 GCH1-TH-SGCE-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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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 in sequence data 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.

LOVD 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 https://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *GCH1* exons 1 and 3 but not exon 2) to MRC Holland: info@mrcholland.com.

Table 1. P099-D1 GCH1-TH-SGCE-PRRT2

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*GCH1*	*TH*	*SGCE*	*PRRT2*
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 00797-L00463	5q				
136 «	**PRRT2 probe** 22454-L31630					**Exon 1**
142 «	**PRRT2 probe** 22453-L30130					**Exon 4**
148	**GCH1 probe** 22452-L03894		**Upstream**			
154 ±	**SGCE probe** 07004-L32095				**Exon 3**	
160 «	**PRRT2 probe** 22455-L32056					**Exon 2**
165	Reference probe 13816-L28133	2q				
172	**SGCE probe** 07892-L07706				**Exon 9**	
178	**GCH1 probe** 03139-L02607		**Exon 2**			
185	**TH probe** 03144-L03587			**Exon 3**		
192	**SGCE probe** 07005-L07151				**Exon 4**	
202	Reference probe 11000-L24654	4q				
210	**TH probe** 03145-L02613			**Exon 4**		
217 «	**PRRT2 probe** 22466-L32057					**Exon 2**
222	**SGCE probe** 03382-L32053				**Exon 1**	
229	**GCH1 probe** 03141-L02609		**Exon 5**			
239	**TH probe** 03146-L14800			**Exon 8**		
247 ±	**SGCE probe** 07003-L26246				**Exon 2**	
255	Reference probe 08728-L26718	9q				
270	**TH probe** 03147-L32096			**Exon 12**		
279	**SGCE probe** 07009-L32097				**Exon 11**	
285	**GCH1 probe** 03686-L32059		**Exon 6**			
292	**SGCE probe** 03384-L32060				**Exon 5**	
301	**TH probe** 03148-L32061			**Exon 14**		
310	Reference probe 13275-L14608	1p				
319	**SGCE probe** 03385-L02778				**Exon 7**	
326 «	**PRRT2 probe** 22458-L31632					**Exon 3**
337	**SGCE probe** 07002-L06608				**Exon 1**	
346	Reference probe 14980-L16716	6q				
355 ±	**SGCE probe** 03386-L02779				**Exon 10**	
364	**GCH1 probe** 15131-L16901		**Exon 4**			
372	**SGCE probe** 19656-L26248				**Exon 6**	
379	**SGCE probe** 21289-L07705				**Exon 8**	
391	Reference probe 01795-L01358	13q				
401	**SGCE probe** 07931-L06613				**Exon 8**	
420	**GCH1 probe** 22461-L03586		**Exon 1**			
426	**TH probe** 22456-L32196			**Exon 1**		
433	**GCH1 probe** 22462-L02608		**Exon 3**			
445	Reference probe 13363-L29187	15q				

[a] See section Exon numbering on page 2 for more information.
± The following probes have a validated SNP on their ligation site: 154 nt probe, rs138616225; 247 nt probe, rs11548284; 355 nt probe, rs148126317. In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.
« 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.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. Target and flanking probes arranged according to chromosomal location

Table 2a. *GCH1*

Length (nt)	MLPA probe	*GCH1* exon[a]	Ligation site[b] NM_000161.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*164-166 (Exon 1)*		
148	22452-L03894	Upstream (Exon 1)	186 nt before exon 1	CGCCCTTTTCCT-TCCCTCCCTGCG	0.6 kb
420	22461-L03586	Exon 1	362-363	ATAACGAGCTGA-ACCTCCCTAACC	37.1 kb
178	03139-L02607	Exon 2	571-572	AAGGACATAGAC-ATGTTTTCCATG	5.6 kb
433	22462-L02608	Exon 3	628-629	GTCCATATTGGT-TATCTTCCTAAC	12.6 kb
364	15131-L16901	Exon 4	61 nt before exon 4	GTTTTATGAGGA-AGGCTTATCAAT	1.4 kb
229	03141-L02609	Exon 5	753-754	CACGGAAGCCTT-GCGGCCTGCTGG	1.8 kb
285	03686-L32059	Exon 6	906-907	CCTGACTCTCAT-TAGGAGCTGAGC	
		stop codon	*914-916 (Exon 6)*		

The exon numbering used in previous versions of this product description can be found in between brackets.

Table 2b. *TH*

Length (nt)	MLPA probe	*TH* exon[a]	Ligation site[b] NM_199292.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*30-32 (Exon 1)*		
426	22456-L32196	Exon 1	69-70	AGGCCAAGGGCT-TCCGCAGGGCCG	1.9 kb
185	03144-L03587	Exon 3	234-235	TCATTGGGCGCA-GGCAGAGCCTCA	1.2 kb
210	03145-L02613	Exon 4	480-481	GGCCCGCCCAGA-GGCCGCGAGCTG	1.7 kb
239	03146-L14800	Exon 8	887-888	CTGGAGGCCTTT-GCTTTGCTGGAG	1.2 kb
270	03147-L32096	Exon 12	1261-1262	CGGGCTGTGTAA-GCAGAACGGGGA	1.4 kb
301	03148-L32061	Exon 14	1477-1478	CTCACGCATCCA-GCGCCCCTTCTC	
		stop codon	*1614-1616 (Exon 14)*		

Table 2c. *SGCE*

Length (nt)	MLPA probe	*SGCE* exon[a]	Ligation site[b] NM_003919.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*36-38 (Exon 1)*		
222	03382-L32053	Exon 1	82-83	CTGTGCTTGGAC-GGGACAGGGTCG	0.6 kb
337	07002-L06608	Exon 1	564 nt after exon 1	GAAATCTCGGTT-TTCTTCGGTTTT	25.6 kb
247 ±	07003-L26246	Exon 2	181-180 reverse	ATGGGTATACAT-TCCGATCGGAGT	1.5 kb
154 ±	07004-L32095	Exon 3	329-328 reverse	CGAAGCCATCCA-GGTCGGTCTGGG	5.2 kb
192	07005-L07151	Exon 4	206 nt after exon 4	AACTCAGATAGG-AAGCCAAACAGA	4.3 kb
292	03384-L32060	Exon 5	595-596	TCTTGGCGCAGT-GAAAAATGTGTG	15.4 kb
372	19656-L26248	Exon 6	721-722	GGTTGGTGCAGA-TGTCCCGTTTTC	2.7 kb
319	03385-L02778	Exon 7	972-973	AAAGCAGAGACT-ATTACACGGATT	0.9 kb
401	07931-L06613	Exon 8	96 nt before exon 8	GTGACAATGTCA-GCATTTCCACAT	0.1 kb
379	21289-L07705	Exon 8	1098-1099	TGCAAACACCAG-AGTAAGTGTCTT	0.9 kb
172	07892-L07706	Exon 9	1263-1264	ATGATAGCACAA-ACATGCCATTGA	10.1 kb
355 ±	03386-L02779	Exon 10	1316-1315 reverse	GTCTGCTGTTGG-GGAATCTGAGTC	3.2 kb
279	07009-L32097	Exon 11	1342-1341 reverse	TTCTTCAGGGAT-ACCATTTACCTG	
		stop codon	*1347-1349 (Exon 11)*		

Table 2d. *PRRT2*

Length (nt)	MLPA probe	*PRRT2* exon[a]	Ligation site[b] NM_145239.3	Partial sequence[c] (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
217 «	22466-L32057	Exon 2	535-536	TCCAAACCAGAA-GTGAGCAAAGAG	0.4 kb
160 «	22455-L32056	Exon 2	919-920	TCTCCCCGAGGT-AGCCTGAGCCGC	0.6 kb
326 «	22458-L31632	Exon 3	1140-1141	GGTAGCCAAGCT-CTTAAGCATCGT	0.2 kb
142 «	22453-L30130	Exon 4	1218-1219	AGTGTATAAGTG-AGGGGCTCTGCC	
		stop codon	*1217-1219 (Exon 4)*		

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.

ᶜ Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± The following probes have a validated SNP on their ligation site: 154 nt probe, rs138616225; 247 nt probe, rs11548284; 355 nt probe, rs148126317. In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.

« 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related products

For related products, see the product page on our website.

References

- De Lonlay P et al. (2000). Tyrosine hydroxylase deficiency unresponsive to L-dopa treatment with unusual clinical and biochemical presentation. *J Inherit Metab Dis*. 23:819-825.
- Gu D et al. (2002). Evidence of multiple causal sites affecting weight in the IGF2-INS-TH region of human chromosome 11. *Hum Genet*. 110:173-181.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*. 30:e57.
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Selected publications using P099 GCH1-TH-SGCE-PRRT2

- Ceravolo R et al. (2013). Expanding the clinical phenotype of DYT5 mutations: Is multiple system atrophy a possible one? *Neurology*. 81:301-302.
- Grunewald A et al. (2008). Myoclonus-dystonia: significance of large SGCE deletions. *Hum Mutat*. 29:331-332.
- Huang CL et al. (2010). Large SGCE deletion contributes to Taiwanes myoclonus-dystonia syndrome. *Parkinsonism Relat Disord*. 16:585-589.
- Shi WT et al. (2015). Han Chinese patients with dopa-responsive dystonia exhibit a low frequency of exonic deletion in the GCH1 gene. *Genet Mol Res*. 14:11185-11190.
- Steinberger D et al. (2007). Utility of MLPA in deletion analysis of GCH1 in dopa-responsive dystonia. *Neurogenetics*. 8:51-55.

P099 product history	
Version	*Modification*
D1	Probes for *PRRT2* have been added, four reference probes have been replaced and two reference probes have been removed. In addition, 11 probes have been adjusted in length.
C3	One reference probe has been added, one flanking probe has been removed and one probe length has been adjusted.
C2	Two reference probes have been replaced and the control fragments have been adjusted (QDX2).
C1	Probe for *GCH1* exon 4 and 3 reference probes have been added, one old reference probe has been removed.
B2	Two DNA denaturation control fragments (D-fragments) at 88 and 96 nt have been added.
B1	Probes for *SGCE* and one additional probe for *GCH1* exon 1 have been added. In addition, six reference probes have been replaced and one reference probe has been removed.
A1	First release.

Implemented changes in the product description
Version D1-02 – 15 May 2025 (05P) - Product description adapted to a new template. - Related SALSA MLPA products section replaced with a reference to the product page. - Exon numbering and ligation site of the 148 nt GCH1 probe adjusted. *Version D1-01 — 21 July 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). - Various minor textual or layout changes. - Ligation sites of the probes targeting the *GCH1*, *TH* and *SGCE* genes updated according to new versions of the NM_ reference sequences. - SNP information about the 154 nt probe changed. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - P099 Product History corrected; version B2 added and modification version B1 corrected. *Version C3-02 – 30 January 2019 (01P)* - Changes compared to previous version were incorrect and are adjusted. From: One reference probe has been replaced and one probe has a small change in length. To: One reference probe has been added, one flanking probe removed and one probe has a small change in length. - Small changes of probe lengths in Table 1 and 2 (from version C3-01 onwards) in order to better reflect the true lengths of the amplification products. *Version C3-01 – 05 July 2018 (01P)* - Product description restructured and adapted to a new template. - Several references were removed, and six new references were added.

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