Product Description
SALSA® MLPA® Probemix P079-A4 OTC

To be used with the MLPA General Protocol.

Version A4
As compared to version A3, one reference probe has been replaced. For complete product history see page 6.

Catalogue numbers:
- **P079-025R:** SALSA MLPA Probemix P079 OTC, 25 reactions.
- **P079-050R:** SALSA MLPA Probemix P079 OTC, 50 reactions.
- **P079-100R:** SALSA MLPA Probemix P079 OTC, 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 P079 OTC is a **research use only (RUO)** assay for the detection of deletions or duplications in the *OTC* gene, which is associated with Ornithine Transcarbamylase Deficiency (OTCD).

OTCD is characterized by a frequent inborn error of the urea cycle, a partially dominant X-linked trait causing hyperammonemia and orotic aciduria. Missense, nonsense, and frameshift mutations in the gene ornithine carbamoyltransferase (*OTC*) lead to OTCD. The protein encoded by this gene is a nuclear-encoded mitochondrial matrix enzyme that catalyses the second step of the urea cycle in mammals. Severe deficiency or total absence of *OTC* results in the accumulation of ammonia and other precursor metabolites during the first few days of life.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK154378/.

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 *OTC* exon numbering used in this P079-A4 OTC product description is the exon numbering from the LRG_846 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. 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
The SALSA MLPA Probemix P079-A4 OTC contains 20 MLPA probes with amplification products between 147 and 382 nucleotides (nt). This includes ten probes for the *OTC* gene, one probe for each exon. In addition, ten reference probes are included that detect locations on the X-chromosome. 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 of the MLPA technique using 16 DNA samples from healthy individuals of the same sex 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 different unrelated individuals who are from families without a history of Ornithine Transcarbamylase Deficiency. It is recommended to use samples of the same sex to facilitate interpretation. 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. 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 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:

Copy Number status: Male samples	Final ratio
Normal	0.80 < FR < 1.20
Deletion	FR = 0
Duplication	1.65 < FR < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	Final ratio
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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 the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software 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. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) 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: 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.
- 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: 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 *OTC* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P079 OTC.
- 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 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.

***OTC* mutation database**

https://databases.lovd.nl/shared/genes/OTC. 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 SNVs and unusual results (e.g., a duplication of *OTC* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P079-A4 OTC

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*OTC*
64-105	Control fragments – see table in probemix content section for more information		
147	Reference probe 07668-L07374	Xp22	
154	**OTC probe** 02632-L02099		**Exon 1**
161	Reference probe 06457-L06570	Xp22	
184	**OTC probe** 02634-L02101		**Exon 3**
202	**OTC probe** 02636-L02103		**Exon 5**
209	Reference probe 01361-L01009	Xp21	
229	**OTC probe** 02638-L02105		**Exon 7**
238 *	Reference probe 17673-L21755	Xq25	
247	Reference probe 01277-L00833	Xq27	
256	**OTC probe** 02640-L02107		**Exon 9**
274	**OTC probe** 02633-L02100		**Exon 2**
283	Reference probe 07661-L07367	Xp11	
301	**OTC probe** 02635-L02102		**Exon 4**
320	**OTC probe** 02637-L02104		**Exon 6**
336	Reference probe 14801-L13868	Xq11	
346	**OTC probe** 02639-L02106		**Exon 8**
355	Reference probe 01281-L00338	Xq25	
364	**OTC probe** 02641-L02108		**Exon 10**
373	Reference probe 01282-L00965	Xq22	
382	Reference probe 01887-L01456	Xq28	

[a] See section Exon numbering on page 1 for more information.
* New in version A4.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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

Length (nt)	SALSA MLPA probe	*OTC* exon[a]	Ligation site NM_000531.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*94-96 (Exon 1)*		
154	02632-L02099	Exon 1	10-11	TGAACACATTTC-TTAGTTTTTAGG	14.7 kb
274	02633-L02100	Exon 2	219-218, reverse	TTTAGAGTGAGA-AGGTCACGGCCC	2.5 kb
184	02634-L02101	Exon 3	364-365	AGAAAAGAAGTA-CTCGAACAAGAT	11.6 kb
301	02635-L02102	Exon 4	461-462	TGTGAATGAAAG-TCTCACGGACAC	20.0 kb
202	02636-L02103	Exon 5	591-592	CTGTCAGATTTG-TACCATCCTATC	2.3 kb
320	02637-L02104	Exon 6	720-719, reverse	ATTCCGAATTTC-GCTGCGCTCATC	5.1 kb
229	02638-L02105	Exon 7	786-787	AGTGTAACCAAG-TTGGCAGAGCAG	0.1 kb
346	02639-L02106	Exon 8	839-840	GCTGACAAATGA-TCCATTGGAAGC	3.0 kb
256	02640-L02107	Exon 9	986-987	TGCCTCTGACTG-GACATTTTTACA	9.2 kb
364	02641-L02108	Exon 10	1136-1135, reverse	GCTTCTGGAGCT-GAGGTGAGTAAT	
		stop codon	*1156-1158 (Exon 10)*		

[a] See section Exon numbering on page 1 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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 P079 OTC

- Bijarnia-Mahay S et al. (2018). Urea cycle disorders in India: clinical course, biochemical and genetic investigations, and prenatal testing. *Orphanet J Rare Dis*, 13(1), 1-12.
- Brassier A et al. (2015). Long-term outcomes in Ornithine Transcarbamylase deficiency: a series of 90 patients. *Orphanet J Rare Dis*. 10:58.
- Cavicchi C et al. (2018). Late-onset N-acetylglutamate synthase deficiency: report of a paradigmatic adult case presenting with headaches and review of the literature. *Int J Mol Sci*, 19(2), 345.
- Choi JH et al. (2015). Clinical outcomes and the mutation spectrum of the OTC gene in patients with ornithine transcarbamylase deficiency. *J Hum Genet*. 60:501-7.
- Lee JH et al. (2014). OTC Gene in Ornithine Transcarbamylase Deficiency: Clinical Course and Mutational Spectrum in Seven Korean Patients. *Pediatr Neurol*. 51:354-9.
- Lu D et al. (2020). Clinical and molecular characteristics of 69 Chinese patients with ornithine transcarbamylase deficiency. *Orphanet J Rare Dis*, 15(1), 1-13.
- Makris G et al. (2021). Clinical and structural insights into potential dominant negative triggers of proximal urea cycle disorders. *Biochimie*, 183, 89-99.
- Micleaa D et al. (2019). Genomic study via chromosomal microarray analysis in a group of Romanian patients with obesity and developmental disability/intellectual disability. *J Pediatr Endocrinol Metab*, 32(7), 667-674.Storkanova G et al. (2013). Ornithine carbamoyltransferase deficiency: molecular characterization of 29 families. *Clin Genet*. 84:552-9.
- Yokoi K et al. (2018). Exonic duplication of the OTC gene by a complex rearrangement that likely occurred via a replication-based mechanism: a case report. *BMC Med Genet*, 19(1), 1-6.

P079 product history	
Version	*Modification*
A4	One reference probe has been replaced.
A3	Five reference probes have been replaced and nine reference probes have been removed.
A2	The 88, 96, 100, and 105 nt control fragments have been included.
A1	First release.

Implemented changes in the product description
Version A4-01– 03 May 2021 (04P) - 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 *OTC* gene updated according to new version of the NM_ reference sequence. *Version 13 – 02 November 2017 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Minor textual and layout changes throughout the document. *Version 12 – 27 November 2015 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

- References updated.
- Manufacturer's address adjusted.

Version 11 – 12 August 2015 (54)

- Various minor textual changes.
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.
- "Peak area" replaced with "peak height".

Version 10 (48)

- Warning added in Table 1, 222 nt probe 02898-L04200.

Version 09 (48)

- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added

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