# Product Description
# SALSA® MLPA® Probemix P455-A1 LZTR1

To be used with the MLPA General Protocol.

**Version A1**

**Catalogue numbers:**
- **P455-025R:** SALSA MLPA Probemix P455 LZTR1, 25 reactions.
- **P455-050R:** SALSA MLPA Probemix P455 LZTR1, 50 reactions.
- **P455-100R:** SALSA MLPA Probemix P455 LZTR1, 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 P455 LZTR1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *LZTR1* gene, which is associated with schwannomatosis.

Schwannomatosis (MIM 162091), the third major form of neurofibromatosis, is a late-onset tumour predisposition disorder that is clinically and genetically distinct from neurofibromatosis types 1 (MIM 162200) and 2 (MIM 101000). In approximately 50% of the schwannomatosis cases, germline mutations in *SMARCB1* have been identified (Smith et al. 2012). Genetic analysis of the schwannomas showed that mutation of *SMARCB1* is often followed by loss of heterozygosity at the 22q region and subsequent mutation of the *NF2* gene. Together, these three events result in biallelic loss of the *SMARCB1* and *NF2* tumour suppressor genes in the schwannomas. In 22q-related schwannomatosis cases without constitutional *SMARCB1* mutations, Piotrowski identified germline mutations in *LZTR1* (Piotrowski et al. 2014). Mutations in *LZTR1* may account for up to 80% of the schwannomatosis cases lacking mutations in *SMARCB1*.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK487394/.

**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 *LZTR1* exon numbering used in this P455-A1 LZTR1 product description is the exon numbering from the LRG_989 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 P455-A1 LZTR1 contains 45 MLPA probes with amplification products between 124 and 500 nucleotides (nt). This includes 20 probes for the *LZTR1* gene, one probe for each exon with the exception of exons 4 and 17 and two probes for exon 21. Furthermore, this probemix also contains 11 flanking probes targeting the 22q11 and 22q12 chromosomal regions surrounding *LZTR1* gene. In addition, 14 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 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 different unrelated individuals who are from families without a history of schwannomatosis. 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 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 | 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 *LZTR1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P455 LZTR1.
- 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.

***LZTR1* mutation database**

https://databases.lovd.nl/shared/genes/LZTR1. 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 *LZTR1* exons 8 and 10 but not exon 9) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P455-A1 LZTR1

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | |
|---|---|---|---|---|
| | | Reference | *LZTR1* | Flanking |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 124 | Reference probe 18709-L21056 | 5q | | |
| 131 | Reference probe 16316-L22397 | 3q | | |
| 136 | Reference probe 10995-L11666 | 4q | | |
| 153 ¬ | **THAP7 probe** 20047-L27123 | | | Telomeric |
| 161 ¬ | **KLHL22 probe** 20041-L05815 | | | Centromeric |
| 166 | **LZTR1 probe** 19994-L27073 | | **Exon 6** | |
| 172 | **LZTR1 probe** 19995-L27440 | | **Exon 10** | |
| 178 | **LZTR1 probe** 19996-L27075 | | **Exon 15** | |
| 184 ¬ | **RIMBP3C probe** 20042-L05796 | | | Telomeric |
| 190 | Reference probe 08838-L08898 | 2p | | |
| 196 | Reference probe 18049-L22439 | 16q | | |
| 202 | **LZTR1 probe** 19997-L27076 | | **Exon 2** | |
| 214 | **LZTR1 probe** 19999-L27078 | | **Exon 8** | |
| 220 Ж | **LZTR1 probe** 20000-SP0909-L27079 | | **Exon 21** | |
| 226 ¬ | **CRKL probe** 20043-L20997 | | | Centromeric |
| 232 | Reference probe 16428-L25931 | 18q | | |
| 244 | **LZTR1 probe** 20001-L27442 | | **Exon 19** | |
| 250 | **LZTR1 probe** 20002-L27081 | | **Exon 12** | |
| 256 Ж | **LZTR1 probe** 20003-SP0910-L27443 | | **Exon 21** | |
| 265 | **LZTR1 probe** 20004-L27083 | | **Exon 1** | |
| 275 ¬ | **SNAP29 probe** 20044-L23315 | | | Centromeric |
| 280 | Reference probe 13350-L26120 | 9q | | |
| 288 | Reference probe 13393-L26813 | 6q | | |
| 301 Ж | **LZTR1 probe** 20005-SP0911-L27084 | | **Exon 5** | |
| 310 | **LZTR1 probe** 20006-L27085 | | **Exon 11** | |
| 318 | **LZTR1 probe** 20007-L27086 | | **Exon 20** | |
| 326 | **LZTR1 probe** 20008-L27087 | | **Exon 13** | |
| 334 Ж ¬ | **THAP7 probe** 20048-SP0915-L27124 | | | Telomeric |
| 346 ¬ | **MYH9 probe** 18630-L23987 | | | Telomeric |
| 355 | Reference probe 05991-L05416 | 20p | | |
| 364 | **LZTR1 probe** 20009-L27088 | | **Exon 7** | |
| 373 | **LZTR1 probe** 20010-L27089 | | **Exon 18** | |
| 390 Ж | **LZTR1 probe** 20012-SP0912-L27750 | | **Exon 14** | |
| 400 ¬ | **CRKL probe** 20045-L16112 | | | Centromeric |
| 409 | Reference probe 08906-L20708 | 11p | | |
| 417 | Reference probe 13817-L15311 | 2q | | |
| 427 ¬ | **HIC2 probe** 20046-L15009 | | | Telomeric |
| 436 | **LZTR1 probe** 20013-L27092 | | **Exon 9** | |
| 444 Ж | **LZTR1 probe** 20014-SP0913-L27093 | | **Exon 16** | |
| 454 | **LZTR1 probe** 20015-L27094 | | **Exon 3** | |
| 463 ¬ | **THAP7 probe** 20049-L27125 | | | Telomeric |
| 474 ¬ | **LARGE probe** 12461-L13462 | | | Telomeric |
| 481 | Reference probe 14909-L05359 | 18p | | |
| 494 | Reference probe 19137-L26747 | 21q | | |
| 500 | Reference probe 19675-L26275 | 4p | | |

[a] See section Exon numbering on page 1 for more information.

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.

¬ 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.

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. *LZTR1* probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | *LZTR1* exon[a] | Ligation site NM_006767.4 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 161 ¬ | 20041-L05815 | *KLHL22* | | TCTTCGATGTTG-TGCTGGTGGTGG | 392.1 kb |
| 275 ¬ | 20044-L23315 | *SNAP29* | | GTATCCACTTAC-CTGTATCATCCA | 36.8 kb |
| 226 ¬ | 20043-L20997 | *CRKL* Exon 1 | | GGTTCGACTCCT-CGGACCGCTCCG | 32.0 kb |
| 400 ¬ | 20045-L16112 | *CRKL* Exon 3 | | ATTGCCGAAGTC-CAGCTTTCTGCA | 32.6 kb |
| | | | | | |
| | | *start codon* | *76-78 (Exon 1)* | | |
| 265 | 20004-L27083 | Exon 1 | 257-258 | CCCGCCCTGCGA-CGAGTTCGTGGG | 0.5 kb |
| 202 | 19997-L27076 | Exon 2 | 316-317 | AAGATGCCATTT-ATGTATTTGGTG | 2.8 kb |
| 454 | 20015-L27094 | Exon 3 | 359-360 | GCTCAATGACCT-CCTGCGGTTCGA | 2.3 kb |
| | *No Probe* | *Exon 4* | | | |
| 301 Ж | 20005-SP0911-L27084 | Exon 5 | 1 nt and 34 nt after exon 5 | ATTGAAGGACGG-33 nt spanning oligo-GGGTCCTGGGTG | 0.7 kb |
| 166 | 19994-L27073 | Exon 6 | 603-604 | GTCGCTAGGTCA-GCCCATGGGGCC | 0.8 kb |
| 364 | 20009-L27088 | Exon 7 | 14 nt before exon 7 | GTCCTCACTGGT-CTGTCCTAATAC | 0.8 kb |
| 214 | 19999-L27078 | Exon 8 | 764-765 | ATCTTGCTGCAA-CTTCCCCGTGGC | 1.2 kb |
| 436 | 20013-L27092 | Exon 9 | 868-869 | GTACCCCCAGGT-GGACACGCATCC | 0.7 kb |
| 172 | 19995-L27440 | Exon 10 | 1224-intron 10 | CCTGCCTCGGAG-GTACAGGCTGGG | 0.5 kb |
| 310 | 20006-L27085 | Exon 11 | 1269-1270 | GTCATCTCGGAC-GCCATGTACATC | 0.8 kb |
| 250 | 20002-L27081 | Exon 12 | 1350-1351 | TCCTGTTACCCT-AAATGCACGCTG | 0.3 kb |
| 326 | 20008-L27087 | Exon 13 | 1455-1456 | CAGGGCCACGTA-GCCATTGTCACA | 0.1 kb |
| 390 Ж | 20012-SP0912-L27750 | Exon 14 | 17 nt before exon 14 and 1534-1535 | GGTGTCCTTGAG-27 nt spanning oligo-AGGAGGCCGCCC | 0.5 kb |
| 178 | 19996-L27075 | Exon 15 | 1741-1742 | AACTGGCACTGA-GCTTCCAGTTGT | 0.3 kb |
| 444 Ж | 20014-SP0913-L27093 | Exon 16 | 1883-1884 and 1922-1923 | GAACTTCGTGGT-39 nt spanning oligo-GTTCGAGCGCCT | 1.1 kb |
| | *No Probe* | *Exon 17* | | | |
| 373 | 20010-L27089 | Exon 18 | 2150-2151 | CTGCAGCTACTT-TGAAGCCATGTT | 0.7 kb |
| 244 | 20001-L27442 | Exon 19 | 2303-2304 | CAGCTACTTGTT-TGCGGCCCCCTA | 0.3 kb |
| 318 | 20007-L27086 | Exon 20 | 2472-2473 | ATTGTGCACCAG-TTCACCAAGGTC | 0.5 kb |
| 220 Ж | 20000-SP0909-L27079 | Exon 21 | 2705-2706 and 2732-2733 | GTGCACCTGCCA-27 nt spanning oligo-CCAAAGAGAGCT | 1.4 kb |
| 256 Ж | 20003-SP0910-L27443 | Exon 21 | 4120-4121 and 4146-4147 | TGGCTCCTGCCA-26 nt spanning oligo-CCCTCCTGGAGG | 1.9 kb |
| | | *stop codon* | *2596-2598 (Exon 21)* | | |
| | | | | | |
| 463 ¬ | 20049-L27125 | *THAP7* Exon 3 | | CTTTCTCCAAGT-TGCGCCGGACAA | 1.2 kb |
| 153 ¬ | 20047-L27123 | *THAP7* Exon 1 | | TCTTTCCCAGAT-GCCGCGTCACTG | 0.2 kb |
| 334 Ж ¬ | 20048-SP0915-L27124 | *THAP7* Exon 1 | | CGCAGCAATGGC-29 nt spanning oligo-AGAGAATCGGCT | 443.1 kb |
| 427 ¬ | 20046-L15009 | *HIC2* | | GTTCCAGCAGAT-CTTGGACTTCAT | 117.7 kb |
| 184 ¬ | 20042-L05796 | *RIMBP3C* | | CTGGGCCCAAGG-CCTAATAGGTGA | 12.2 **Mb** |
| 474 ¬ | 12461-L13462 | *LARGE* | | ATCTCCAGTGCT-AAGAACTCAGGC | 2.5 **Mb** |
| 346 ¬ | 18630-L23987 | *MYH9* | | CAGCTGAACTCG-TTTGAGCAGCTG | |

[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.

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.
¬ 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.

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.

## Related SALSA MLPA probemixes

| | |
|---|---|
| P044 NF2 | Contains probes for the *NF2* gene, involved in Neurofibromatosis type 2. |
| P081/P082 NF1 | Contains probes for the *NF1* gene, involved in Neurofibromatosis type 1. |
| P250 DiGeorge | Contains probes for the 22q11 region, used for primary screening of this region. |
| P258 SMARCB1 | Contains probes for the *SMARCB1* gene. Deletions and mutations in *SMARCB1* are associated with malignant rhabdoid tumours and schwannomatosis. |
| P295 SPRED1 | Contains probes for the *SPRED1* gene, involved in Neurofibromatosis type I-like syndrome. |

## References


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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.
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- Smith MJ et al. (2012). Frequency of SMARCB1 mutations in familial and sporadic schwannomatosis. *Neurogenetics*, 13(2), 141-145.
- 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 P455 LZTR1


- Godel T et al. (2018). Dorsal root ganglia volume differentiates schwannomatosis and neurofibromatosis 2. *Ann Neurol*, 83(4), 854-857.
- Kehrer-Sawatzki H et al. (2018). Phenotypic and genotypic overlap between mosaic NF2 and schwannomatosis in patients with multiple non-intradermal schwannomas. *Hum Genet*, 137(6), 543-552.
- Kehrer-Sawatzki H et al. (2018). Co-occurrence of schwannomatosis and rhabdoid tumor predisposition syndrome 1. *Mol Genet Genom Med*, 6(4), 627-637.
- Smith MJ et al. (2017). Revisiting neurofibromatosis type 2 diagnostic criteria to exclude LZTR1-related schwannomatosis. *Neurology*, 88(1), 87-92.


| **P455 product history** | |
|---|---|
| *Version* | *Modification* |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| Version A1-01 – 14 May 2021 (04P)<br>- Product description rewritten and adapted to a new template.<br>- Ligation sites of the probes targeting the *LZTR1* gene updated according to new version of the NM_ reference sequence.<br>*Version 02 – 12 October 2017 (55)*<br>- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).<br>*Version 01 – 05 February 2015 (54)*<br>- Not applicable, new document. |

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