# Product Description
# SALSA® MLPA® Probemix P130-A5/P131-B2 CCM

To be used with the MLPA General Protocol.

**Version A5**
As compared to version A4, five reference probes have been replaced and four reference probes have been removed. For complete product history see pages 8
**Version B2**
As compared to version B1, three reference probes have been replaced and one probe length has been adjusted. For complete product history see pages 9.

Catalogue numbers:
- **P130-025R:** SALSA MLPA probemix P130 CCM mix-1, 25 reactions.
- **P130-050R:** SALSA MLPA probemix P130 CCM mix-1, 50 reactions.
- **P130-100R:** SALSA MLPA probemix P130 CCM mix-1, 100 reactions.

- **P131-025R:** SALSA MLPA probemix P131 CCM mix-2, 25 reactions.
- **P131-050R:** SALSA MLPA probemix P131 CCM mix-2, 50 reactions.
- **P131-100R:** SALSA MLPA probemix P131 CCM mix-2, 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 P130 and P131 CCM are a **research use only (RUO)** assay for the detection of deletions or duplications in the *KRIT1 (CCM1)*, *CCM2* and *PDCD10 (CCM3)* genes, which are associated with Cerebral cavernous malformations (CCMs).

Cerebral cavernous malformations (CCMs) are vascular lesions of the brain that may lead to hemorrhage, seizures, and neurologic deficits. Most are linked to loss-of-function mutations in one of the aforementioned genes, that can either occur as sporadic events or are inherited in an autosomal dominant pattern with incomplete penetrance. Familial forms originate from germline mutations, often have multiple intracranial lesions that grow in size and number over time, and cause an earlier and more severe presentation.

The *KRIT1* gene (20 exons) spans ~47 kb of genomic DNA and is located on 7q21.2, ~92 Mb from the p-telomere. The *CCM2* gene (11 exons) spans ~76 kb of genomic DNA and is located on 7p13, ~45 Mb from the p-telomere. The *PDCD10* gene (9 exons) spans ~51 kb of genomic DNA and is located on 3q26.1, 167 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/ NBK1116/.

**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 *KRIT1 (CCM1)*, *CCM2* and *PDCD10 (CCM3)* exon numbering used in this P130-A5 CCM mix-1 and P131-B2 CCM mix-2 product description is the exon numbering from the LRG_650, LRG_664, LRG_651 and the NG_012964.1, NG_016295.1 and NG_008158.1 sequences. The *CCM2* exon numbering for the 301 nt probe has changed. From description version B2-01 onwards, we have adopted the NCBI exon numbering that is present in the LRG for this gene. The exon numbering used in previous versions of this product description can be found in between brackets in Table 2. 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 or NG 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 P130-A5 CCM mix-1 contains 34 MLPA probes with amplification products between 148 and 454 nt. The SALSA MLPA Probemix P131-B2 CCM mix-2 contains 31 MLPA probes with amplification products between 131 and 408 nt.

The P130 probemix contains probes for nine of the 20 exons of *KRIT1* and one probe for every exon of the *CCM2* gene (two probes for exon 3). In addition, 13 reference probes are included in this probemix, detecting several different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

The P131 probemix contains probes for nine of the 20 exons of *KRIT1* and one probe for every exon of the *PDCD10* gene. One probe is located in intron 2 of the *PDCD10* gene and detects an alternative exon 2 in transcription variant 2 (NM_145859.1). In addition, 12 reference probes are included in this probemix, detecting several different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.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 Cerebral cavernous malformations (CCMs). 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 *KRIT1 (CCM1)*, *CCM2* and *PDCD10 (CCM3)* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemixes P130 and P131.
- 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.

***KRIT1 (CCM1)*, *CCM2* and *PDCD10 (CCM3)* mutation databases**

https://databases.lovd.nl/shared/genes/KRIT1; https://databases.lovd.nl/shared/genes/CCM2; https://databases.lovd.nl/shared/genes/PDCD10. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 *PDCD10* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

## Tabel 1a. SALSA MLPA Probemix P130-A5 CCM Mix-1

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | |
|---|---|---|---|---|
| | | Reference | *CCM2* | *KRIT1* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 148 | Reference probe 10663-L11245 | 6p | | |
| 154 | **CCM2** probe 04364-L03784 | | **Exon 7** | |
| 160 | **KRIT1** probe 08100-L28600 | | | **Exon 8** |
| 166 * | Reference probe 16058-L18232 | 9p | | |
| 171 | **KRIT1** probe 04351-L03771 | | | **Exon 14** |
| 178 | **CCM2** probe 04359-L28450 | | **Exon 3** | |
| 184 | Reference probe 11262-L28451 | 19q | | |
| 190 « | **CCM2** probe 05083-L04503 | | **Upstream** | |
| 196 | **KRIT1** probe 04343-L03763 | | | **Exon 6** |
| 202 * | Reference probe 19414-L25829 | 3p | | |
| 209 | **CCM2** probe 04365-L28407 | | **Exon 8** | |
| 220 | Reference probe 06746-L26706 | 8q | | |
| 226 | **KRIT1** probe 04593-L28452 | | | **Exon 3** |
| 233 | **CCM2** probe 04360-L28453 | | **Exon 3** | |
| 241 | Reference probe 07030-L28177 | 14q | | |
| 257 | **KRIT1** probe 04594-L03773 | | | **Exon 16** |
| 265 | **CCM2** probe 04366-L04205 | | **Exon 9** | |
| 281 | Reference probe 17450-L26854 | 16p | | |
| 291 | **KRIT1** probe 04355-L03775 | | | **Exon 18** |
| 301 « | **CCM2** probe 05084-L04504 | | **Exon 2** | |
| 319 * | Reference probe 17528-L20415 | 15q | | |
| 331 | **KRIT1** probe 04347-L28459 | | | **Exon 10** |
| 337 | **CCM2** probe 04367-L28458 | | **Exon 10** | |
| 346 * | Reference probe 13441-L14896 | 17p | | |
| 355 | Reference probe 11614-L12374 | 12p | | |
| 369 | **KRIT1** probe 04357-L28457 | | | **Exon 20** |
| 378 | **CCM2** probe 04362-L28456 | | **Exon 5** | |
| 387 | Reference probe 01522-L21320 | 10p | | |
| 402 | **KRIT1** probe 04349-L28454 | | | **Exon 12** |
| 409 | **CCM2** probe 04368-L28455 | | **Exon 11** | |
| 418 * | Reference probe 21261-L29869 | 20p | | |
| 436 | **CCM2** probe 04596-L28601 | | **Exon 4** | |
| 445 | **CCM2** probe 04597-L03783 | | **Exon 6** | |
| 454 | Reference probe 12526-L23849 | 4q | | |

[a] See section Exon numbering on page 2 for more information.

* New in version A5.
« 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. Single probe aberration(s) must be confirmed by another method.

## Table 1b. SALSA MLPA Probemix P131-B2 CCM Mix-2

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | |
|---|---|---|---|---|
| | | Reference | *KRIT1* | *PDCD10* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 131 ¥ | Reference probe 00797-L25925 | 5q | | |
| 136 | **PDCD10** probe 04393-L03791 | | | **Exon 1** |
| 142 | **KRIT1** probe 04340-L28602 | | **Exon 2** | |
| 148 * | Reference probe 10663-L11245 | 6p | | |
| 154 | Reference probe 05277-L26403 | 14q | | |
| 165 | **PDCD10** probe 04398-L28485 | | | **Exon 5** |
| 172 | **KRIT1** probe 04350-L28484 | | **Exon 13** | |
| 178 | Reference probe 03179-L04606 | 9p | | |
| 188 | **KRIT1** probe 20616-L28504 | | **Exon 11** | |
| 198 | **PDCD10** probe 04589-L28483 | | | **Exon 7** |
| 206 | **KRIT1** probe 08099-L28595 | | **Exon 4** | |
| 213 | **PDCD10** probe 04587-L28481 | | | **Intron 2** |
| 221 * | Reference probe 14791-L16502 | 11p | | |
| 232 | **PDCD10** probe 20617-L28478 | | | **Exon 8** |
| 239 | **PDCD10** probe 04395-L28477 | | | **Exon 2** |
| 247 | Reference probe 10808-L11455 | 4q | | |
| 253 | **KRIT1** probe 04344-L28503 | | **Exon 7** | |
| 261 | Reference probe 07819-L23623 | 1q | | |
| 274 | **PDCD10** probe 04588-L03797 | | | **Exon 6** |
| 281 | Reference probe 17450-L26854 | 16p | | |
| 292 | **KRIT1** probe 04354-L28475 | | **Exon 17** | |
| 300 * | Reference probe 10662-L22500 | 2p | | |
| 310 | **PDCD10** probe 04396-L03794 | | | **Exon 3** |
| 323 | **KRIT1** probe 04346-L28473 | | **Exon 9** | |
| 337 | Reference probe 03195-L02652 | 17q | | |
| 346 | **PDCD10** probe 04401-L03799 | | | **Exon 9** |
| 362 | **KRIT1** probe 04590-L28471 | | **Exon 5** | |
| 372 | Reference probe 16852-L19646 | 18q | | |
| 382 | **PDCD10** probe 04397-L03795 | | | **Exon 4** |
| 400 | **KRIT1** probe 04591-L28470 | | **Exon 19** | |
| 408 | Reference probe 14405-L22478 | 12q | | |

[a] See section Exon numbering on page 2 for more information.

* New in version B2.
¥ Changed in version B2. Minor alteration, no change in sequence detected.

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.

## P130-A5/P131-B2 probes arranged according to chromosomal location

Tabel 2a. *KRIT1*

| Length (nt) | | SALSA MLPA probe | *KRIT1* Exon[a] | Ligation site NM_194456.1 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|---|
| P130 | P131 | | | | | |
| | | | *Start Codon* | *785-787 (exon 5)* | | |
| | | No Probe | Exon 1 | | | |
| | 142 | 04340-L28602 | Exon 2 | 217-218 | CACCCATTTAGT-TGAATGTAGCGG | 0.6 kb |
| 226 | | 04593-L28452 | Exon 3 | 567-568 | CTAAATGGCAGT-TGTACATCTCCC | 0.8 kb |
| | 206 | 08099-L28595 | Exon 4 | 656-657 | GTCTGTCTCATA-ATTTGTAAACGT | 2.0 kb |
| | 362 | 04590-L28471 | Exon 5 | 838-839 | ATTCGTCCAAAG-AATACTGCCAGT | 1.0 kb |
| 196 | | 04343-L03763 | Exon 6 | 1009-1010 | GTAGTAGAAACC-ACCAAACCAATT | 3.3 kb |
| | 253 | 04344-L28503 | Exon 7 | 1095-1096 | TGGAGAGAAGAT-GGGCAGAGAAGC | 1.3 kb |
| 160 | | 08100-L28600 | Exon 8 | 1229-1230 | CCAGTACTCATT-TTGCTACACTTA | 0.9 kb |
| | 323 | 04346-L28473 | Exon 9 | 1365-1366 | CATAAATCCTGC-ATATGCTACTGA | 0.7 kb |
| 331 | | 04347-L28459 | Exon 10 | 1572-1573 | CTACTCAAAAAT-CCAAATACCTAA | 0.3 kb |
| | 188 | 20616-L28504 | Exon 11 | 1698-1699 | AGAATTACTAAG-CCGTCTTCTCAG | 7.9 kb |
| 402 | | 04349-L28454 | Exon 12 | 1833-1834 | CAATCCAAACCT-TTTAAATGGACA | 0.8 kb |
| | 172 | 04350-L28484 | Exon 13 | 1962-1963 | AAGATCTCCATT-AAATATTTGTGA | 2.9 kb |
| 171 | | 04351-L03771 | Exon 14 | 2080-2081 | GGGTCATATCGT-TCTGTTGAATTG | 0.9 kb |
| | | No Probe | Exon 15 | | | |
| 257 | | 04594-L03773 | Exon 16 | 2436-2437 | TGATGCTAAGCT-GATAACATTGGC | 8.0 kb |
| | 292 | 04354-L28475 | Exon 17 | 2553-2554 | TGTTACCAAACT-GAAAAGTAAGGC | 0.6 kb |
| 291 | | 04355-L03775 | Exon 18 | 2699-2700 | ATGGAGCAGCAT-TTTTCACAGGAC | 11.9 kb |
| | 400 | 04591-L28470 | Exon 19 | 2839-2840 | AAGTATGGTTGT-TTTATGTGGCAA | 0.7 kb |
| 369 | | 04357-L28457 | Exon 20 | 3060-3061 | TCCGTGAAAGAT-TTCTTAAAATAT | |
| | | | *Stop Codon* | *2993-2995 (exon 20)* | | |

Table 2b. *CCM2* (P130-A5)

| Length (nt) | SALSA MLPA probe | *CCM2* Exon[a] | Ligation site in NM_031443.4 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *Start Codon* | *134-136 (exon 1)* | | |
| 190 | 05083-L04503 | Upstream | 248 nt before exon 1 | GGCAGGCAGACT-TTATAACAGAGA | 0.9 kb |
| | No Probe | Exon 1 | | | |
| 301 | 05084-L04504 | Exon 2 (1) | 2 nt after exon 2 | GGGCAAGAAGGT-GAGCGTGCGCGG | 37.9 kb |
| 178 | 04359-L28450 | Exon 3 | 194-195 | TTAAACGAGTAT-TCCTAAAAGGTG | 0.1 kb |
| 233 | 04360-L28453 | Exon 3 | 321-322 | GAGCGACTATAT-TGAGAAGGAGGT | 25.5 kb |
| 436 | 04596-L28601 | Exon 4 | 373-374 | CCAGGATACCTG-AATCCCTCCAGT | 0.6 kb |
| 378 | 04362-L28456 | Exon 5 | 522-523 | CGGGGAGGATAT-CATCCTCAGGGT | 3.9 kb |
| 445 | 04597-L03783 | Exon 6 | 652-653 | TGTGCGGAAAGT-TCCAGAGGCCTC | 1.4 kb |
| 154 | 04364-L03784 | Exon 7 | 792-793 | CTTCCAGGTTGT-TTACACGGAGTC | 2.9 kb |
| 209 | 04365-L28407 | Exon 8 | 896-897 | ACAGATGACTCT-TCTACAAAAGTG | 0.8 kb |
| 265 | 04366-L04205 | Exon 9 | 967-968 | GATGTGGGTGGT-GCATCACCCCAC | 0.9 kb |
| 337 | 04367-L28458 | Exon 10 | 1133-1134 | CTATCCACGAGT-TCTGCATCAACC | 1.5 kb |
| 409 | 04368-L28455 | Exon 11 | 1235-1236 | ACTTCGAGAACT-TCCTGGAGACCA | |
| | | *Stop Codon* | *1466-1468 (exon 11)* | | |

Table 2c. *PDCD10* (P131-B2)

| Length (nt) | SALSA MLPA probe | *PDCD10* Exon[a] | Ligation site in NM_007217.4 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *Start Codon* | *485-487 (exon 3)* | | |
| 136 | 04393-L03791 | Exon 1 | 186-187 | CTTGGGCTTGGT-TCTGGCAGGCGG | 0.4 kb |
| 239 | 04395-L28477 | Exon 2 | 286-287 | TACGGATTCAGT-TCCAGTTTAATG | 8.9 kb |
| 213 | 04587-L28481 | Intron 2 | 5.1 kb before exon 3; NM_145859.1; 106-107 | GACTAACAGCAT-TTTGGCATGAGA | 5.3 kb |
| 310 | 04396-L03794 | Exon 3 | 537-538 | CACATCCATGGT-TTCTATGCCCCT | 15.2 kb |
| 382 | 04397-L03795 | Exon 4 | 615-616 | AGCCCAGACACT-GAGAGCCGCTTT | 7.8 kb |
| 165 | 04398-L28485 | Exon 5 | 672-673 | ACAAGACATCAT-TATGAAAATTTT | 1.4 kb |
| 274 | 04588-L03797 | Exon 6 | 783-784 | AGAGCCAGAATT-CCAAGACCTAAA | 8.1 kb |
| 198 | 04589-L28483 | Exon 7 | 934-935 | AATGTCTTCAAG-AAATATCAATAC | 0.3 kb |
| 232 | 20617-L28478 | Exon 8 | 981-982 | CCAAAAGAAAGA-ATTTGTAAAGTA | 2.9 kb |
| 346 | 04401-L03799 | Exon 9 | 1071-1072 | AAGTGCCAACCG-ACTAATTCATCA | |
| | | *Stop Codon* | *1121-1123 (exon 9)* | | |

[a] See section Exon numbering on page 2 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 P130 CCM mix-1/131 CCM mix-2


- Scimone C. et al (2015). Detection of novel mutation in CCM3 causes familial cerebral caverous Malformations. *J. Mol. Neurosci*. 57, 400–403.
- Mondejar R. et al (2014). Mutation prevalance of cerebral cavernous malformation genes in Spanish patients. *PLoS One* 9 (1): e86286.
- Spiegler S. et al (2014). High mutation detection rates in cerebral cavernous malformation upon stringent inclusion criteria: one-third of probands are minors. *Mol. Genet. and Genomic Med*. 2(2): 176-185.
- D'Angelo R. et al (2013). Sporadic cerebral cavernous malformations: report of further mutations in CCM genes in 40 Italian patients. *Biomed. Res*. Int. 2013; 459253.
- Mosca L. et al (2012). De novo MGC4607 gene heterozygous missense variants in an child with multiple cerebral cavernous malformations. *J. Mol. Neurosci*. 47(3): 475-480.


| P130 product history | |
|---|---|
| *Version* | *Modification* |
| A5 | Five reference probes have been replaced and four reference probes have been removed. |
| A4 | One reference probe has been removed. |
| A3 | One flanking probe and one reference probe has been removed and 14 reference probes have been replaced. |
| A2 | The 88, 96, 100 and 105 nt control fragments (QDX2) have been included. |
| A1 | First release. |

| **P131 product history** | |
|---|---|
| *Version* | *Modification* |
| B2 | Three reference probes have been replaced and one probe length has been adjusted. |
| B1 | One target probe has been removed, in addition six reference probes have been replaced and six reference probes have been removed. |
| A2 | The 88, 96, 100 and 105 nt control fragments (QDX2) have been included. |
| A1 | First release. |

**Implemented changes in the product description**

*Version A5-01 (P130)/B2-01 (P131)* – 28 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 *CCM2* and *PDCD10* genes updated according to new versions of the NM_ reference sequences.
- Exon numbering of the 301nt probe detecting the *CCM2* gene has been changed in Table 1 and 2.

*Version A4-01 (P130)/B1-01 (P131) – 22 March 2019 (01P)*
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version.
- Small changes in Table 1 and Table 2.

*Version 12 – 02 September 2015 (55)*
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new pictures included).
- Exon numbering of the *KRIT/CCM1* gene has been changed in Table 1 and 2.
- Exon numbering of the *CCM2* gene has been changed in Table 1 and 2.
- Exon numbering of the *PDCD10* gene has been changed in Table 1 and 2.
- Ligation sites of the probes targeting the *KRIT1*, *CCM2* and *PCD10* gene updated according to the recent NM_reference sequence.
- Various minor textual and layout changes.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- References for SALSA® MLPA® probemix P130/P131 updated.



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