# Product Description SALSA® MLPA® Probemix P015-F2 MECP2

To be used with the MLPA General Protocol.

**Version F2.** Compared to version F1, two reference probes have been replaced and the 118 nt Y fragment has been removed. For complete product history see page 11.

**Catalogue numbers:**
- **P015-025R:** SALSA MLPA probemix P015 MECP2, 25 reactions.
- **P015-050R:** SALSA MLPA probemix P015 MECP2, 50 reactions.
- **P015-100R:** SALSA MLPA probemix P015 MECP2, 100 reactions.

To be used in combination with a SALSA® MLPA® reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

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

**Precautions and warnings:** For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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.

**Intended use:** The SALSA MLPA probemix P015 MECP2 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *MECP2* gene, in order to confirm a potential cause and clinical diagnosis for classic and atypical Rett syndrome, and for *MECP2* duplication syndrome. This assay can also be used for the detection of deletions or duplications in the human *NTNG1* and *CDKL5* genes, in order to confirm a potential cause and clinical diagnosis for atypical Rett syndrome, and for the detection of deletions or duplications in the human *ARX* and *CDKL5* genes, in order to confirm a potential cause and clinical diagnosis for X-linked intellectual disability syndrome. This product can also be used for molecular genetic testing of at-risk family members. This assay is for use with human DNA extracted from peripheral blood.

Deletions or duplications detected with the P015 MECP2 probemix must be confirmed by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in *MECP2*, *CDKL5*, *ARX* and *NTNG1* genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of the aforementioned genes. Not all exons of *CDKL5*, *ARX* and *NTNG1* genes are covered. The P189 is available for deletion or duplication analysis of other *CDKL5*, *ARX* and *NTNG1* exons. This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test must be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

**Clinical background:** Rett syndrome is a dominant X-linked brain disorder characterized by microcephaly, loss of achieved psychomotor abilities, intellectual disability, and autistic behaviours. The prevalence of Rett syndrome is approximately 1 in 10.000 live female births. Rett syndrome is usually caused by mutations in the *MECP2* gene, although patients with mutations in two other genes, *CDKL5* and *FOXG1*, may also exhibit a Rett-like phenotype. While loss-of-function mutations in *MECP2* result in Rett syndrome, gain-of-function mutations are associated with MECP2 duplication syndrome which occurs almost exclusively in males. MECP2 duplication syndrome and Rett syndrome share overlapping clinical phenotypes including intellectual disability, motor deficits, epilepsy, hypotonia, and progressive spasticity.

Atypical Rett syndrome is the early onset seizure variant of Rett syndrome and is characterized by seizures in the first months of life with subsequent development of Rett syndrome features. It has an estimated

prevalence of 1 in 45.000. *MECP2* alterations in Rett syndrome patients comprise mainly point mutations (approximately 80% in classical and 40% in atypical cases), while deletions or duplications are less common (8% in classical and 3% in atypical cases). Approximately 6.5-10% of patients with atypical Rett syndrome have large deletions in *CDKL5* (Rett syndrome database RettBASE).
One report described a patient with atypical Rett syndrome who presented with early onset of epileptic seizures (not infantile spasms) and a *de novo* translocation in intron 6 of *NTNG1* gene (Borg et al. 2005). Please note that MLPA will not detect balanced translocations.
Mutations in *CDKL5* mainly cause X-linked early infantile epileptic encephalopathy, also called X-linked infantile spasm syndrome. *ARX* gene mutations account for 9.5% of all cases of X-linked intellectual disability syndromes, including X-linked lissencephaly with abnormal genitalia, X-linked infantile spasm syndrome, and Partington syndrome (neurological disorder with movement problems). Since these genes are involved in multiple syndromes and are covered in multiple probemixes, Table 1 is provided to give an overview.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1497/ and https://www.ncbi.nlm.nih.gov/books/NBK1284/.

**Table 1**: Overview on the genes and probemixes related to (atypical) Rett syndrome.

| Condition | Genes | Probemix and coverage | Remarks |
|---|---|---|---|
| **Classic Rett syndrome** | *MECP2* | P015*: Each exon<br>P374: Exon 2, 3, 4<br>P245*: Exon 1, 3, 4 | MECP2 probe exon 2 in P374 is different from P015.<br>MECP2 probes in P245 are identical to P015 and P374 (different length). |
| **MECP2 duplication syndrome** | *MECP2* | P015*: Each exon<br>P374: Exon 2, 3, 4<br>P245*: Exon 1, 3, 4 | MECP2 probe exon 2 in P374 is different from P015.<br>MECP2 probes in P245 are identical to P015 and P374 (different length). |
| **Atypical Rett syndrome** | *MECP2* | P015*: Each exon<br>P374: Exon 2, 3, 4<br>P245*: Exon 1, 3, 4 | MECP2 probe exon 2 in P374 is different from P015.<br>MECP2 probes in P245 are identical to P015 and P374 (different length). |
| | *CDKL5* | P189*: Each exon<br>P015*: Exons 3, 6, 9, 10<br>P259: Exon 2, 17, 20 | CDKL5 probes in P015 and in P259 are identical to P189 (different length). |
| | *NTNG1* | P189*: Each exon<br>P015*: Exons 2, 3, 5, 6 | NTNG1 probes in P015 are identical to P189 except for exon 2 (different length). |
| **FOXG1 syndrome** | *FOXG1* | P189*: Each exon<br>P075: Each exon + upstream<br>P395: Each exon + upstream | FOXG1 probes in P189 are identical to P075 and P395 (different length).<br>One FOXG1 exon 1 probe is different between P075 and P395. |
| **X-linked intellectual disability syndrome** | *CDKL5* | P189*: Each exon<br>P015*: Exons 3, 6, 9, 10<br>P259: Exon 2, 17, 20 | CDKL5 probes in P015 and in P259 are identical to P189 (different length). |
| | *ARX* | P189*: Each exon<br>P015*: Exons 1, 5<br>P106: Exons 1, 2, 4 | ARX probes in P015 and P106 are identical to P189 (different length). |

*IVD. All other for Research Use Only (RUO).

**Gene structure:**
The *MECP2* gene (4 exons) spans ~76 kb of genomic DNA on chromosome Xq28. The status of *MECP2* LRG_764 is pending, but available at http://www.lrg-sequence.org, and identical to GenBank NG_007107.2.
The *CDKL5* gene (21 exons) spans ~228 kb of genomic DNA on chromosome Xp22.13.
The *ARX* gene (5 exons) spans ~12 kb of genomic DNA on chromosome Xp21.3.
The *NTNG1* gene (6 exons) spans ~345 kb of genomic DNA on chromosome 1p13.3.

**Transcript variants**:
The *MECP2* LRG_764 is identical to RefSeq transcript NM_004992.3 (10241 nt, coding sequence 227-1687): https://www.ncbi.nlm.nih.gov/gene/4204. *MECP2* MLPA probes are indicated according to NM_004992.3 in Table 3.
Transcript variant NM_003159.2 (3434 nt, coding sequence 254-3346) for *CDKL5* is described: https://www.ncbi.nlm.nih.gov/gene/6792. This sequence is a reference standard in the RefSeqGene project. *CDKL5* MLPA probes are indicated according to NM_003159.2 in Table 3.
Transcript variant NM_139058.2 (2885 nt, coding sequence 212-1900) for *ARX* is described: https://www.ncbi.nlm.nih.gov/gene/170302. This sequence is a reference standard in the RefSeqGene project. *ARX* MLPA probes are indicated according to NM_139058.2 in Table 3.
Transcript variant NM_014917.3 (6199 nt, coding sequence 808-2124) for *NTNG1* is described: https://www.ncbi.nlm.nih.gov/gene/22854. This sequence is a reference standard in the RefSeqGene project. *NTNG1* MLPA probes are indicated according to NM_014917.3 in Table 3.

**Exon numbering:** The exon numbering used in this P015-F2 MECP2 product description is the exon numbering from the RefSeq transcript NM_004992.3 for *MECP2*, which is identical the LRG_764 sequence. For *CDKL5* the exon numbering used is from the RefSeq transcript variant NM_003159.2, for *ARX* from the RefSeq transcript NM_139058.2, and for *NTNG1* from the RefSeq transcript NM_014917.3. The exon numbering and NM sequences used are from 07/2018, but can be changed by NCBI after the release of the product description.

**Probemix content:** The P015-F2 probemix contains 46 probes with amplification products between 130 nt and 467 nt. The P015-F2 probemix contains 17 probes for the *MECP2* gene, covering each exon. Furthermore, several probes are present for genes in close proximity to *MECP2*. One of these probes is located within the pseudo autosomal region 2 (*PAR2*). The P015-F2 probemix contains four *CDKL5* probes, two *ARX* probes, and four *NTNG1* probes. More probes for the *CDKL5*, *ARX* and *NTNG1* genes are present in the P189 CDKL5/ARX/FOXG1 probemix. In addition, 10 reference probes are included in this probemix, detecting several different autosomal chromosomal locations. 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.mlpa.com.

| Length (nt) | Name |
|---|---|
| 64-70-76-82 | Q-fragments (Only visible with <100 ng sample DNA) |
| 88-96 | D-fragments (Low signal of 88 or 96 fragment indicates incomplete denaturation) |
| 92 | Benchmark fragment |
| 100 | X-fragment (X chromosome specific) |
| 105 | Y-fragment (Y chromosome specific) |

**MLPA technique:** The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.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 from peripheral blood, 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:** All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of Rett syndrome. It is recommended to use reference and patient 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.

**Positive control DNA samples:** MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Female sample ID number NA23676 from the Coriell Institute has been tested at MRC-Holland and can be used as positive control sample to detect a heterozygous whole gene duplication of *MECP2*. The quality of cell lines can change, therefore samples should be validated before use.

**Performance characteristics:** The frequency of *MECP2* deletions or duplications in classic Rett syndrome is 8% and 3% in atypical cases. Approximately 6.5-10% of patients with atypical Rett syndrome have large deletions in *CDKL5* (Rett syndrome database RettBASE). The frequency of *ARX* deletions or duplications is ~1% in X-linked intellectual disability syndrome cases https://www.omim.org/entry/300382. No deletions or duplications in the *NTNG1* gene have been described so far. The analytical sensitivity and specificity for the detection of deletions or duplications in the *MECP2*, *CDKL5*, *ARX*, and *NTNG1* genes is very high and can be considered >99% (based on a 2000-2016 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

**Data analysis:** Coffalyser.Net software must 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.mlpa.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 expected results for the probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). The same results can be expected for the X-chromosome-specific probes in female samples. For the X-chromosome-specific probes in male samples, expected copy numbers are 1 (normal), 0 (deletion) or 2 (duplication).

The standard deviation of all probes in the reference samples should be <0.10. When this criteria is fulfilled, the following cut-off values for the dosage quotient (DQ) of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

| Copy Number status | | Dosage quotient |
|---|---|---|
| **Autosomal sequences<br>X-chromosome sequences in female** | **X-chromosome sequences in male** | |
| Normal | Normal | 0.80 < DQ < 1.20 |
| Homozygous deletion | Deletion | DQ = 0 |
| Heterozygous deletion | - | 0.40 < DQ < 0.65 |
| Heterozygous duplication | - | 1.30 < DQ < 1.65 |
| Heterozygous triplication/<br>Homozygous duplication | Duplication | 1.75 < DQ < 2.15 |
| Ambiguous copy number | Ambiguous copy number | All other values |

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *MECP2*, *CDKL5*, *ARX*, *NTNG1* genes. 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 (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 in some cases 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.
- Deletion of a probe's recognition sequence on the X-chromosome will lead to a complete absence of the corresponding probe amplification product in males, whereas female heterozygotes are recognizable by a 35-50% reduction in relative peak height.

**Limitations of the procedure:**

- In most populations, the major cause of genetic defects in the *MECP2*, *CDKL5*, *ARX*, and *NTNG1* genes are small (point) mutations, none of which will be detected by using SALSA MLPA probemix P015-MECP2.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- Not all exons of the *CDKL5*, *ARX* and *NGNT1* genes are covered in the P015 MECP2 probemix.

**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 one or 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.

**RettBASE mutation database:** http://mecp2.chw.edu.au/mecp2/index.php. We strongly encourage users to deposit positive results in the RettBASE mutation 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 SNPs and unusual results (e.g., a duplication of *MECP2* exons 1 and 3 but not exon 2) to MRC-Holland: info@mlpa.com.

# Table 2. SALSA MLPA P015-F2 MECP2 probemix

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | | | |
|---|---|---|---|---|---|---|
| | | Reference | MECP2 Xq28 | CDKL5 Xp22.13 | ARX Xp21.3 | NTNG1 1p13 |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | | |
| 130 | Reference probe 00797-L21056 | 5q31 | | | | |
| 136 « ¬ | **GDI1 probe** 16875-L19669 | | Xq28 | | | |
| 143 | **CDKL5 probe** 06466-L06567 | | | **Exon 10** | | |
| 149 | **NTNG1 probe** 18447-L24143 | | | | | **Exon 2** |
| 154 ~ | **MECP2 probe** 18442-L24243 | | **Exon 4b** | | | |
| 161 « | **ARX probe** 18440-L24338 | | | | **Exon 1** | |
| 166 | Reference probe 08222-L24146 | 10q26 | | | | |
| 172 « ¬ | **SLC6A8 probe** 01879-L24147 | | Xq28 | | | |
| 178 « ¬ | **IRAK1 probe** 10835-L24148 | | Xq28 | | | |
| 182 « | **MECP2 probe** 10836-L24339 | | **Exon 1** | | | |
| 190 | **CDKL5 probe** 06458-L23617 | | | **Exon 3** | | |
| 196 | **MECP2 probe** 10839-L23618 | | **Exon 3** | | | |
| 202 « | **MECP2 probe** 03409-L16570 | | **Exon 1** | | | |
| 208 | Reference probe 09865-L08705 | 13q32 | | | | |
| 214 « | **MECP2 probe** 03770-L13387 | | **Upstream** | | | |
| 221 ¬ | **F8 probe** 06288-L05892 | | Xq28 | | | |
| 229 ~ | **MECP2 probe** 18441-L12494 | | **Exon 4b** | | | |
| 235 « | **ARX probe** 06455-L21229 | | | | **Exon 5** | |
| 241 ¬ | **L1CAM probe** 07051-L14553 | | Xq28 | | | |
| 247 * | Reference probe 05959-L05376 | 7p11 | | | | |
| 254 « | **MECP2 probe** 03768-L23833 | | **Exon 2** | | | |
| 260 « ± | **MECP2 probe** 01769-L23834 | | **Exon 4b** | | | |
| 266 * | Reference probe 10728-L22588 | 6p12 | | | | |
| 274 ~ | **MECP2 probe** 01768-L13824 | | **Exon 4b** | | | |
| 280 | Reference probe 16434-L24149 | 18q21 | | | | |
| 286 | **CDKL5 probe** 06462-L24150 | | | **Exon 6** | | |
| 292 ~ | **MECP2 probe** 18444-L24151 | | **Exon 4b** | | | |
| 299 | Reference probe 16621-L24152 | 1q32 | | | | |
| 305 « | **MECP2 probe** 02002-L24153 | | **Exon 1** | | | |
| 312 | **NTNG1 probe** 06487-L24154 | | | | | **Exon 5** |
| 319 ¬ | **DKC1 probe** 18761-L24340 | | Xq28 | | | |
| 338 | Reference probe 09776-L24156 | 15q21 | | | | |
| 346 ~ | **MECP2 probe** 01347-L24157 | | **Exon 4b** | | | |
| 356 | **MECP2 probe** 10841-L24158 | | **Exon 4b** | | | |
| 365 | **MECP2 probe** 01348-L24159 | | **Exon 3** | | | |
| 373 « | **MECP2 probe** 01349-L24160 | | **Exon 2** | | | |
| 383 ¬ | **IDH3G probe** 01887-L23933 | | Xq28 | | | |
| 391 | **MECP2 probe** 14737-L24161 | | **Exon 3** | | | |
| 400 | **CDKL5 probe** 06465-L05991 | | | **Exon 9** | | |
| 409 | Reference probe 17462-L21218 | 12p13 | | | | |
| 418 « | **MECP2 probe** 18446-L23620 | | **Exon 4b** | | | |
| 427 | **NTNG1 probe** 06488-L23934 | | | | | **Exon 6** |
| 438 | **NTNG1 probe** 06483-L24162 | | | | | **Exon 3** |
| 448 « ¬ | **FLNA probe** 04138-L24163 | | Xq28 | | | |
| 457 ¬ | **VAMP7 probe** 01094-L24164 | | Xq28-**PAR2** | | | |
| 467 | Reference probe 02674-L24165 | 11q22 | | | | |

**(a)** The exon numbering used in this P015-F2 product description is identical for *MECP2* to the exon numbering from the LRG_764 sequence and to the RefSeq transcript NM_004992.3, for *CDKL5* to the RefSeq transcript variant NM_003159.2, for *ARX* to the RefSeq transcript NM_139058.2, and for *NTNG1* to the RefSeq transcript NM_014917.3. Exon numbering used here may differ from literature. Please notify us of any mistakes.

* New in version F2 (from lot F2-0118 onwards).
± SNP rs267608346 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

« 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.
˜ Probe located within, or close to, a hotspot region of the *MECP2* gene in which small deletions occur frequently (Laccone et al. 2004, Huppke et al. 2005). In addition to small deletions, many SNPs are also present in this region, making this probe more prone to false positive deletion results.
¬ 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.

# Table 3. P015 probes arranged according to chromosomal location

Table 3a. *MECP2* / Xq28 region (from telomere to centromere)

| Length (nt) | SALSA MLPA probe | Gene/ Exon[a] | Ligation site[b] | Partial sequence[c] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *q-telomere* | | | *~90.0 kb* |
| 457 ¬ | 01094-L24164 | VAMP7 Exon 8 (**PAR2 region**) | | TGTGGGAAAAGT-GTTTCCATTCTG | 957.0 kb |
| 221 ¬ | 06288-L05892 | F8 | | TTCAGGGAGTCT-GGCCAAGGAAAA | 218.1 kb |
| 319 ¬ | 18761-L24340 | DKC1 | | CATGATGTGCTT-GATGCTCAGTGG | 331.9 kb |
| 136 « ¬ | 16875-L19669 | GDI1 | | CCTGACCATGGA-CGAGGAATACGA | 79.7 kb |
| 448 « ¬ | 04138-L24163 | FLNA | | TGACGGCACGTA-TACAGTGGCCTA | 221.7 kb |
| | | **MECP2** | **NM_004992.3** | | |
| | | *start codon* | *227-229 (ex 2)* | | |
| 214 « | 03770-L13387 | Upstream | 1093 nt before ex 1 | GCAAGAATGTTA-GTTTGCTGTCTG | 0.7 kb |
| 305 « | 02002-L24153 | Exon 1 | 380 nt before ex 1, reverse | GGGACGCCTGTT-TGCGCTGCTCTG | 0.1 kb |
| 202 « | 03409-L16570 | Exon 1 | 318 nt before ex 1 | CATTAATCCTTA-ACATTCAAATTC | 0.4 kb |
| 182 « | 10836-L24339 | Exon 1 | 52-53 | GGGCTGTGGTAA-AAGCCGTCCGGA | 5.3 kb |
| 254 « | 03768-L23833 | Exon 2 | 44 nt before ex 2 | GAAAAAGGTCGT-GCAGCTCAATGG | 0.1 kb |
| 373 « | 01349-L24160 | Exon 2 | 188-189 | GACTCCCCAGAA-TACACCTTGCTT | 59.6 kb |
| 196 | 10839-L23618 | Exon 3 | 47 nt before ex 3 | ACTTGTTCTGCA-GACTGGCATGTT | 0.2 kb |
| 365 | 01348-L24159 | Exon 3 | 385-386 | GCCCACCACTCT-GCTGAGCCCGCA | 0.2 kb |
| 391 | 14737-L24161 | Exon 3 | 582-583 | CTCTGCTGGGAA-GTATGATGTGTA | 0.7 kb |
| 356 | 10841-L24158 | Exon 4b | 32 nt before ex 4b | AGAGCCTCTAAT-TGTTCCTTGTGT | 0.3 kb |
| 229 ~ | 18441-L12494 | Exon 4b | 896-897 | TCCTTGTCAAGA-TGCCTTTTCAAA | 0.3 kb |
| 346 ~ | 01347-L24157 | Exon 4b | 1243-1244 | CTGAAGACCTGT-AAGAGCCCTGGG | 0.2 kb |
| 154 ~ | 18442-L24243 | Exon 4b | 1479-1480 | AGAGGAGAAGAT-GCCCAGAGGAGG | 0.1 kb |
| 274 ~ | 01768-L13824 | Exon 4b | 1622-1623 | TTTCATCCTCCA-TGCCAAGGCCAA | 0.1 kb |
| 292 ~ | 18444-L24151 | Exon 4b | 1682-1683 | CCGAGAGAGTTA-GCTGACTTTACA | 2.2 kb |
| 418 « | 18446-L23620 | Exon 4b | 3871-3872 | TGCTGCCATGAA-CTGTCAAGTGTG | 3.2 kb |
| 260 « ± | 01769-L23834 | Exon 4b | 7036-7037 | CAGTAACACATA-GACTGTGCGCAT | 5.9 kb |
| | | *stop codon* | *1685-1687 (ex 4b)* | | |
| 178 « ¬ | 10835-L24148 | IRAK1 | | TTTATGAAGCTT-TTCCAGGCTCCC | 154.4 kb |
| 241 ¬ | 07051-L14553 | L1CAM | | CAGCGGGTGAAA-ACTACAGTGTCG | 70.3 kb |
| 383 ¬ | 01887-L23933 | IDH3G | | TCCCCGAAACTT-CGCACCCCGTCG | 99.8 kb |
| 172 « ¬ # | 01879-L24147 | SLC6A8 | | ACCCCGCTGGTC-TGCATGGTAAGG | - |

Table 3b. *CDKL5* (chromosome X)

| Length (nt) | SALSA MLPA probe | CDKL5 Exon[a] | Ligation site[b] NM_003159.2 | Partial sequence[c] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *254-256 (ex 2)* | | |
| 190 | 06458-L23617 | Exon 3 | 42 nt before ex 3 | GAGCTTTGTAGT-TTGTATGCGTGC | 69.1 kb |
| 286 | 06462-L24150 | Exon 6 | 573-574 | GCCAAATGGAGT-TCCACCTGAGAA | 8.2 kb |
| 400 | 06465-L05991 | Exon 9 | 913-914 | ATTGACCAACTT-TTTACTATTCAG | 7.3 kb |
| 143 | 06466-L06567 | Exon 10 | 1026-1027 | TCCTCAGTCCTT-GGAAAGAAGATA | - |
| | | *stop codon* | *3344-3346 (ex 21)* | | |

Table 3c. *ARX* (chromosome X)

| Length (nt) | SALSA MLPA probe | ARX Exon[a] | Ligation site[b] NM_139058.2 | Partial sequence[c] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *212-214 (ex 1)* | | |
| 161 « | 18440-L24338 | Exon 1 | 98-99 | AGATCGCAATAA-TATCCGTTATAA | 11.2 kb |
| 235 « | 06455-L21229 | Exon 5 | 1964-1965 | CAGCACCACTCA-AGACCAAATGGA | - |
| | | *stop codon* | *1898-1900 (ex 5)* | | |

Table 3d. *NTNG1* (chromosome 1)

| Length (nt) | SALSA MLPA probe | NTNG1 Exon[a] | Ligation site[b] NM_014917.3 | Partial sequence[c] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *808-810 (ex 2)* | | |
| 149 | 18447-L24143 | Exon 2 | 578-579 | ACAAGTATGTTA-GGCTTCCACCAA | 176.5 kb |
| 438 | 06483-L24162 | Exon 3 | 1606-1607 | GGATAAGGCTGT-TAAGACCAGCCG | 83.1 kb |
| 312 | 06487-L24154 | Exon 5 | 227 nt after ex 5 | TATGACTTTTCT-GACTACTCTTAA | 72.7 kb |
| 427 | 06488-L23934 | Exon 6 | 1907-1908 | GAATGTCTGCGA-CAACGAGCTCCT | - |
| | | *stop codon* | *2122-2124 (ex 6)* | | |

**(a)** The exon numbering used in this P015-F2 MECP2 product description is identical for *MECP2* to the exon numbering of the LRG_764 sequence and to the RefSeq transcript NM_004992.3, for *CDKL5* to the RefSeq transcript variant NM_003159.2, for *ARX* to the RefSeq transcript NM_139058.2, and for *NTNG1* to the RefSeq transcript NM_014917.3.
**(b)** Ligation sites of the P015 MECP2 MLPA probes are indicated according to RefSeq sequence NM_004992.3 for *MECP2* containing 4 exons, for *CDKL5* to the RefSeq sequence NM_003159.2 containing 21 exons, for *ARX* to the RefSeq sequence NM_139058.2 containing 5 exons, and for *NTNG1* to the RefSeq sequence NM_014917.3 containing 6 exons.
**(c)** Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

˜ Probe located within, or close to, a hotspot region of the *MECP2* gene in which small deletions occur frequently (Laccone et al. 2004, Huppke et al. 2005). In addition to small deletions, many SNPs are also present in this region, making this probe more prone to false positive deletion results.
« 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.
± SNP rs267608346 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
¬ 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.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

## Related SALSA® MLPA® probemixes

- P189 CDKL5/ARX/FOXG1: Contains probes for the *CDKL5*, *NTNG1*, *ARX* and *FOXG1* genes.
- P075 TCF4-FOXG1: Contains probes for the *TCF4* and *FOXG1* genes (Pitt-Hopkins syndrome).
- P336 UBE3A: For the detection of small rearrangements in the *UBE3A* gene, involved in Angelman syndrome.
- P395 MEFC2-FOXG1: Contains probes for the *MEFC2* and *FOXG1* genes (mental retardation).
- P106 MRX: Contains probes for various genes that cause X-linked mental retardation when defect.
- ME028 Prader Willi / Angelman syndrome: For the detection of both copy number as well as methylation changes of the Prader Willi / Angelman region.
- P245 Microdeletion Syndromes-1A: Includes probes for *MECP2*.
- P374: Microdeletion Syndromes 8: Includes probes for *MECP2*.
- P259: RPS6KA3 (Coffin-Lowry syndrome): Includes probes for *CDKL5*.

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| **P015 MECP2 Product history** | |
|---|---|
| *Version* | *Modification* |
| F2 | Two reference probes have been replaced, the 118 nt Y fragment has been removed. |
| F1 | Four MECP2 probes (exons 3 and 4) and two additional reference probes have been included. One ARX, one NTNG1 and several reference probes have been replaced. One CDKL5 and one IRAK1 probe have been removed. The 88 and 96 nt denaturation control probes have been replaced (QDX2). |
| E1 | One MECP2 exon 3 probe has been removed. |
| D2 | Several new MECP2 probes have been included and extra probes up- and downstream of MECP2 were added. In addition, some probes for the CDKL5, ARX and NTNG1 genes are included. |
| D1 | Test Lot. |
| C2 | Extra control fragments at 88, 96, 100 and 105 nt have been added. |
| B1 | One extra probe for MECP2 exon 1, three extra MECP2 exon 4 probes and extra reference probes have been included. |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| *Version F2-03 – 18 May 2020 (04)*<br>- Israel added as country with IVD status.<br>*Version F2-02 – 30 July 2018 (04)*<br>- Warning added to Table 3 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.<br>- Selected publications were updated using P015.<br>*Version F2-01 – 01 March 2018 (04)*<br>- Product description restructured and adapted to a new template.<br>- Product description adapted to a new product version (version number changed, changes in Table 2 and Table 3).<br>- Table 1 is included to give an overview of the included genes in different probemixes related to (atypical) Rett syndrome.<br>*Version 15 - 27 January 2017 (55)*<br>- Probe number (182nt MECP2 probe 10836-L24339) adjusted as it was incorrect displayed in previous product description version.<br>*Version 14 - 15 November 2015 (55)*<br>- Lot number added, new pictures included.<br>- CDKL5 exon numbering adjusted.<br>- Various minor textual changes.<br>*Version 13 (53)*<br>- Various minor layout changes.<br>- Updated link for "Database of genomic variants".<br>- "Peak area" replaced with "peak height".<br>*Version 12 (52)*<br>- Reference to previous lot adjusted.<br>*Version 11 (49)*<br>- Product description adapted to a new version (lot number added, small changes in Table 1 and Table 2, small textual changes, new pictures included). |



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|---|---|
| | MRC-Holland bv; Willem Schoutenstraat 1<br>1057 DL, Amsterdam, The Netherlands |
| E-mail | info@mlpa.com (information & technical questions); order@mlpa.com (orders) |
| Phone | +31 888 657 200 |



| | |
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| IVD | EUROPE* CE<br>ISRAEL |
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*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA). The product is for RUO in all other European countries.