Product Description SALSA® MLPA® Probemix P488-B1 RS1

To be used with the MLPA General Protocol.

Version B1. For complete product history see page 6.

Catalogue numbers:
- **P488-025R:** SALSA MLPA Probemix P488 RS1, 25 reactions.
- **P488-050R:** SALSA MLPA Probemix P488 RS1, 50 reactions.
- **P488-100R:** SALSA MLPA Probemix P488 RS1, 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.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.

General information: The SALSA MLPA Probemix P488 RS1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *RS1* gene, which is associated with X-linked juvenile retinoschisis.

X-linked juvenile retinoschisis is a retinal dystrophy that leads to schisis (splitting) of the neural retina leading to reduced visual acuity in affected men. The split in the retina occurs predominantly within the inner retinal layers and is very different from retinal detachment, which is a split between the neural retina and the retinal pigment epithelium. This condition is caused by defects in the *RS1* gene that lead to a dysfunctional retinoschisin protein which causes impaired adhesion of retinal cells. The prevalence of X-linked juvenile retinoschisis is estimated between 1 in 15,000 and 1 in 30,000 (Sikkink et al. 2007).

The *RS1* gene (6 exons) spans ~32.4 kb of genomic DNA and is located on Xp22.13, 18.6 Mb from the p-telomere. The *CDKL5* gene partially overlaps with *RS1*, having exons flanking *RS1* and exons within introns of *RS1* (see Figure 1). This SALSA MLPA Probemix contains probes for both *RS1* and *CDKL5*.

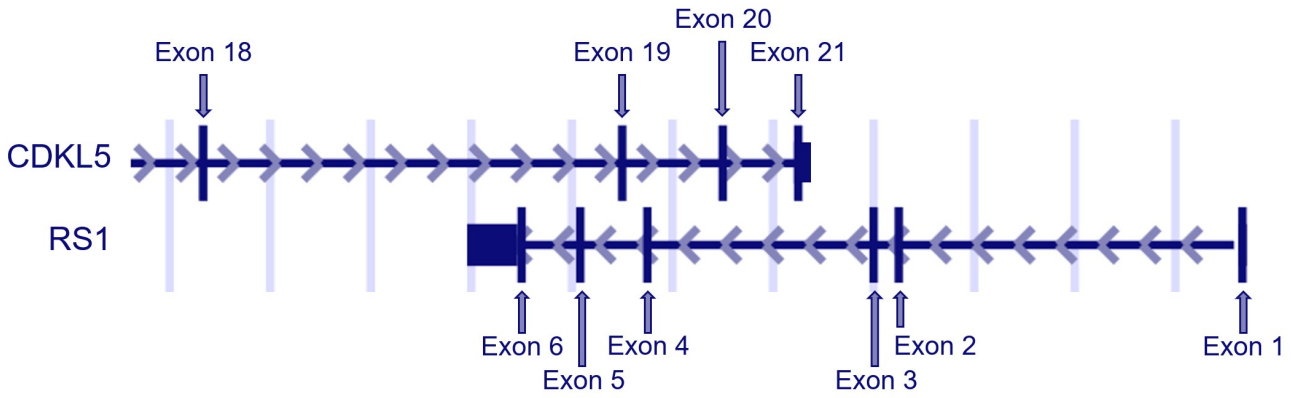

Figure 1. All exons of *RS1* (below, reverse orientation) and exons 18-21 of *CDKL5* (above) displayed in UCSC Genome browser. Exon 19 of *CDKL5* is located in intron 4 of *RS1*, and exons 20 and 21 of *CDKL5* are located in intron 3 of *RS1*.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1222/.

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 *RS1* and *CDKL5* exon numbering used in this P488-B1 RS1 product description is the exon numbering from the LRG_702 sequence and NG_008475.1 sequence, respectively. The exon numbering used has been retrieved on 12/2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P488-B1 RS1 contains 19 MLPA probes with amplification products between 157 and 298 nucleotides (nt). This includes six probes for the *RS1* gene (one probe for each exon of the gene), and four probes for the *CDKL5* gene. In addition, nine reference probes are included that detect other locations on the X-chromosome. The probes targeting *CDKL5* could be informative on the size of the deletion or duplication. Complete probe sequences and the identity of the genes detected by the reference probes are 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 nt and 96 nt 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, 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 unrelated individuals who are from families without a history of hereditary eye or vision abnormalities. 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.

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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.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 standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results:

Copy Number status: Male samples	Dosage quotient
Normal	0.80 < DQ < 1.20
Deletion	DQ = 0
Duplication	1.65 < DQ < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
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. 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *RS1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P488 RS1.
- 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.

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.

***RS1* mutation database:** https://databases.lovd.nl/shared/genes/RS1. 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 SNPs and unusual results (e.g., a duplication of *RS1* exons 4 and 6 but not exon 5) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P488-B1 RS1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*RS1*	*CDKL5*
64-105	Control fragments – see table in probemix content section for more information			
157	Reference probe 20449-L28192	Xp11		
166	Reference probe 04423-L05579	Xp22		
172	**RS1 probe** 21245-L29081		**Exon 1**	
178	Reference probe 05153-L04557	Xq22		
184 Ø	**CDKL5 probe** 21246-L29116			**Exon 20**
190	Reference probe 07653-L07359	Xp11		
195	**RS1 probe** 21247-L29115		**Exon 5**	
211 Ø	**CDKL5 probe** 21255-L31466			**Exon 18**
220	**RS1 probe** 21248-L29086		**Exon 2**	
226	Reference probe 13519-L14318	Xq21		
232 Ø	**CDKL5 probe** 21257-L20734			**Exon 21**
238	Reference probe 08248-L07945	Xq26		
246	**RS1 probe** 21249-L29085		**Exon 6**	
256	**RS1 probe** 21250-L29117		**Exon 3**	
267 Ø	**CDKL5 probe** 21251-L29084			**Exon 19**
274	Reference probe 19689-L26419	Xq23		
283	**RS1 probe** 21254-L29087		**Exon 4**	
291	Reference probe 01370-L01287	Xp21		
298	Reference probe 05614-L05891	Xq28		

a) See Figure 1 and the above section on exon numbering for more information.

Ø Probe detects the *CDKL5* gene, which partially overlaps with the *RS1* gene. *CDKL5* exons 19, 20 and 21 are situated in *RS1* intronic sequences.

Table 2. RS1/CDKL5 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*RS1* exon[a]	Ligation site NM_000330.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*41-43 (Exon 1)*		
172	21245-L29081	Exon 1	2 nt before exon 1 *reverse*	TTACTGAACTGG-AGAGCTGGCCCA	14.5 kb
220	21248-L29086	Exon 2	105-106	CACATTGGGATT-ATCGTCTACCGA	0.9 kb
256	21250-L29117	Exon 3	173-174	ATTGCCAAGGAG-GACCCAATGCTC	3.1 kb
232 Ø	21257-L20734	*CDKL5* gene; Exon 21	NM_003159.2; 3402-3403	CTCATGGAAGAA-CCAATTAACACC	3.1 kb
184 Ø	21246-L29116	*CDKL5* gene; Exon 20	NM_003159.2; 3148-3147 *reverse*	ACCTGGAGTACT-GGATAGGGAGCA	3.1 kb
283	21254-L29087	Exon 4	61 nt before exon 4	TTGTTATTTCAG-TCACCTGGTGCT	1.4 kb
267 Ø	21251-L29084	*CDKL5* gene; Exon 19	NM_003159.2; 72 nt before exon 19 *reverse*	AAACATATTCTA-TTGGCCACTTCT	1.3 kb
195	21247-L29115	Exon 5	44 nt before exon 5	CTTTTTGCAGAC-ATGCACAGGGGG	3.1 kb
		stop codon	*713-715 (Exon 6)*		
246	21249-L29085	Exon 6	1095-1096	ACCACATTCAAG-ACAGAAACATAC	13.1 kb
211 Ø	21255-L31466	*CDKL5* gene; Exon 18	NM_003159.2; 2864-2865	CCAAAAATTCCT-TCTCAGAAATTC	

a) See Figure 1 and the above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Ø Probe detects the *CDKL5* gene, which partially overlaps with the *RS1* gene. *CDKL5* exons 19, 20 and 21 are situated in *RS1* intronic sequences.

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.
- Sikkink SK et al. (2007). X-linked retinoschisis: an update. *J Med Genet.* 44:225-232.
- 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.

P488 Product history	
Version	*Modification*
B1	First release.

Implemented changes in the product description
Version B1-01 — 31 January 2020 (02P) - Not applicable, new document.

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