Product Description SALSA® MLPA® Probemix P379-A2 NRXN1

To be used with the MLPA General Protocol.

Version A2. For complete product history see page 6.

Catalogue numbers:
- **P379-025R:** SALSA MLPA Probemix P379 NRXN1, 25 reactions.
- **P379-050R:** SALSA MLPA Probemix P379 NRXN1, 50 reactions.
- **P379-100R:** SALSA MLPA Probemix P379 NRXN1, 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.

General Information: The SALSA MLPA Probemix P379 NRXN1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *NRXN1* gene, which is associated with Pitt-Hopkins-like syndrome 2.

Pitt-Hopkins-like syndrome 2 is characterised by intellectual disability (mental retardation), wide mouth and distinctive facial features, and intermittent hyperventilation/overbreathing. This syndrome is caused by defects in the *NRXN1* gene on chromosome 2p16.3. Neurexin 1 protein is encoded by the *NRXN1* gene. Neurexins, including Neurexin 1, are cell-surface receptors that bind neuroligins at synapses in the central nervous system. This transsynaptic complex is required for efficient neurotransmission and is involved in the formation of synaptic contacts.

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/

Probemix content: The SALSA MLPA Probemix P379-A2 NRXN1 contains 43 MLPA probes with amplification products between 130 and 449 nt. This includes one probe for each exon of the *NRXN1* gene. A second probe is included for exons 1, 10, 19, 20, 23, and 24. Furthermore two probes are included for an alternative exon present in the beta transcript variant (NM_138735.4). This exon is present in intron 18 of the main alpha2 transcript variant (NM_001135659.2). In addition, 11 reference probes are included and detect 11 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 121 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)

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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: 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 Pitt-Hopkins-like syndrome 2. 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. 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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes 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 for autosomal or pseudo-autosomal chromosomes:

Copy Number status	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 (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 are unlikely to have any relation to the condition tested for.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *NRXN1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P379 NRXN1.
- 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.

Mutation database: https://databases.lovd.nl/shared/genes/NRXN1. We strongly encourage users to deposit positive results in the 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 *NRXN1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P379-A2 NRXN1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*NRXN1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
135	**NRXN1** probe 15861-L17954		**Exon 1**
141	**NRXN1** probe 16305-L18830		**Exon 13**
148	**NRXN1** probe 15863-L17956		**Exon 10**
157	**NRXN1** probe 15864-L18520		**Exon 21**
166	**NRXN1** probe 15865-L18430		**Exon 6**
172	**NRXN1** probe 15866-L17959		**Exon 23**
178	**NRXN1** probe 15867-L17960		**Exon 12**
185	**NRXN1** probe 16657-L19735		**Exon 2**
190	Reference probe 12422-L13423	14q24	
196 «	**NRXN1** probe 15869-L18432		**Intron 18**
202	Reference probe 13339-L14765	18q21	
208	**NRXN1** probe 15871-L18433		**Exon 24**
215	**NRXN1** probe 15872-L17965		**Exon 9**
220	Reference probe 13013-L14179	4q35	
228 Ж	**NRXN1** probe 15873-SP0298-L17966		**Exon 4**
233	**NRXN1** probe 15874-L18434		**Exon 19**
238	Reference probe 05335-L04722	1p21	
247	**NRXN1** probe 15876-L18435		**Exon 15**
256 «	**NRXN1** probe 15877-L17970		**Intron 18**
263	**NRXN1** probe 15878-L18436		**Exon 14**
270	**NRXN1** probe 16659-L19210		**Exon 3**
277	**NRXN1** probe 20622-L18437		**Exon 8**
286	**NRXN1** probe 15880-L18438		**Exon 19**
292	Reference probe 03796-L03237	21q22	
299	**NRXN1** probe 15883-L17976		**Exon 23**
306	**NRXN1** probe 15881-L18439		**Exon 7**
312	**NRXN1** probe 15882-L18440		**Exon 20**
319	Reference probe 14713-L16385	10q25	
336	**NRXN1** probe 15886-L18441		**Exon 20**
346	**NRXN1** probe 15885-L17978		**Exon 18**
355	Reference probe 10086-L10510	8q22	
364	**NRXN1** probe 15887-L17980		**Exon 11**
373	**NRXN1** probe 15888-L17981		**Exon 22**
382	Reference probe 07808-L07538	3p22	
391	**NRXN1** probe 15889-L17982		**Exon 24**
400	**NRXN1** probe 15890-L17983		**Exon 10**
409	**NRXN1** probe 15891-L17984		**Exon 1**
418	**NRXN1** probe 15892-L17985		**Exon 5**
427	**NRXN1** probe 15893-L17986		**Exon 17**
433	Reference probe 06962-L20682	1q31	
439	**NRXN1 probe** 16188-L28435		**Exon 16**
449	Reference probe 11256-L28436	11q21	

« 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.
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.

Note: The exon numbering used in this P379-A2 NRXN1 product description is the exon numbering from the RefSeq transcript NM_001135659.2. The exon numbering and NM sequence used is from 12/2018 but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. NRXN1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*NRXN1* exon	Ligation site NM_001135659.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*1478-1480 (exon 2)*		
409	15891-L17984	Exon 1	135-136	ATGCAAAGGTAA-AAGCACCTCGCT	0.3 kb
135	15861-L17954	Exon 1	463-464	TCTGAAAAGGCA-GCAGCAGTCGCC	3.3 kb
185	16657-L19735	Exon 2	969-970	AGCATCCAAGGA-TACTGGCTCACT	2.2 kb
270	16659-L19210	Exon 3	71 nt before exon 3	TAATTTCTGTGG-TTCTTGGGGCTT	100.6 kb
228 Ж	15873-SP0298-L17966	Exon 4	25 nt before exon 4, 2352-2353	TTTTCCTTTTCT-29 nt spanning oligo-CAACAATGTGGA	3.2 kb
418	15892-L17985	Exon 5	27 nt before exon 5	CCTTGTTTATTG-CTGACTGTGCTC	291.5 kb
166	15865-L18430	Exon 6	102 nt before exon 6	GAACAAGAGAAG-TTAGACTCAGAA	7.7 kb
306	15881-L18439	Exon 7	2554-2555	CACACTGGGAAA-TCGGCTGATTAT	2.2 kb
277 #	20622-L18437	Exon 8	2722-2721 reverse	ATAGCGTGTCCA-ATGCCTGAGTGC	1.1 kb
215	15872-L17965	Exon 9	2 nt before exon 9	GCTTTGTTATCT-AGGTGACAATAT	67.2 kb
148	15863-L17956	Exon 10	2969-2970	GACTTGCCAAGC-AAGGAGATCCTA	0.3 kb
400	15890-L17983	Exon 10	3316-3317	GTGAATGATGGA-GAATGGTATCAT	14.0 kb
364	15887-L17980	Exon 11	3390-3391	GCGTACTCCCTA-CACTGCTCCTGG	7.3 kb
178	15867-L17960	Exon 12	3857-3858	GTGCATATGGCA-TTCTGATGGCAA	2.6 kb
141	16305-L18830	Exon 13	69 nt before exon 13	TGGACTGTAATT-TTATTGGATGTC	22.1 kb
263	15878-L18436	Exon 14	4006-4007	GCTGGCTATAAC-CTCAATGATAAC	8.9 kb
247	15876-L18435	Exon 15	4159-4160	ATCATCACAGAA-CGACGGTATCTT	1.7 kb
439	16188-L28435	Exon 16	4600-4601	AGCAACCTCCAC-ACTGTAAAGATT	23.6 kb
427	15893-L17986	Exon 17	4747-4748	GAAGGCTTTCAA-GGCTGCCTGGCA	6.9 kb
346	15885-L17978	Exon 18	4888-4889	AATCAAGGTGTG-TGCTTGCAACAA	117.8 kb
196 «	15869-L18432	Intron 18	70-71 (NM_138735.4)	TTCTTCTCAGCT-GCCTTTCCGCCG	1.0 kb
256 «	15877-L17970	Intron 18	1029-1030 (NM_138735.4)	GTGCCTATTGCA-ATCTACAGGTCA	109.8 kb
233	15874-L18434	Exon 19	4972-4973	GCTGGGACGACA-TATATCTTTAGC	0.1 kb
286	15880-L18438	Exon 19	5108-5109	GAGTGGACAGTT-CTTCAGGCTTGG	145.4 kb
336	15886-L18441	Exon 20	5179-5180	TTTAATGTTGGG-ACAGATGACATC	0.1 kb
312	15882-L18440	Exon 20	5251-5252	GTTCGTTTCACG-AGGAGTGGTGGC	36.4 kb
157	15864-L18520	Exon 21	5362-5363	CAGCGAATTCCA-TATCGACTTGGT	1.5 kb
373	15888-L17981	Exon 22	5518-5519	GGCTTGAAAGTT-CTGAATATGGCA	109.7 kb
172	15866-L17959	Exon 23	5733-5734	GCAGACCACAGA-TGACATCCTTGT	0.1 kb
299	15883-L17976	Exon 23	53 nt after exon 23	ATCTTTGTTGCA-TGTTATATTCCT	21.5 kb
208	15871-L18433	Exon 24	5965-5966	TACAAGTACAGA-AACCGGGATGAA	0.6 kb
391	15889-L17982	Exon 24	6556-6557	AGTCAGTCCAAT-TGCCAGAGAAGA	
		stop codon	*6119-6121 (exon 24)*		

« 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.
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.
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.

Note: The exon numbering used in this P379-A2 NRXN1 product description is the exon numbering from the RefSeq transcript NM_001135659.2. The exon numbering and NM sequence used is from 12/2018 but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P075 TCF4-FOXG1: Contains probes for the *TCF4* and *FOXG1* genes, involved in Pitt-Hopkins syndrome.
- P297 Microdeletion-2: Contains two probes for *CNTNAP2*.
- P343 Autism-1: Contains probes for the regions 15q11-13, 16p11.2 and 22q13.3 (*SHANK3*), involved in autism.

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.

P379 Product history	
Version	*Modification*
A2	One reference probe was added.
A1	First release.

Implemented changes in the product description

Version A2-01 – 24 December 2018 (01P)
- Product description restructured and adapted to a new template.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 04 – 12 May 2015 (54)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Exon numbering of the NRXN1 gene has been adjusted on page 3 and 4.

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