

Product Description

SALSA® MLPA® Probemix P379-A2 NRXN1

To be used with the MLPA General Protocol.

Version A2

For complete product history see page 7.

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

SALSA® MLPA® Probemix P379 NRXN1 (hereafter: P379 NRXN1) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P379-025R	P379-050R	P379-100R	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		
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A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis

Information regarding 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

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, 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 product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:

Entrez Gene shows transcript variants of each gene: <https://www.ncbi.nlm.nih.gov/gene>

For NM_ mRNA reference sequences: <https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide>

Matched Annotation from NCBI and EMBL-EBI (MANE): <https://www.ncbi.nlm.nih.gov/refseq/MANE>

Exon numbering

The *NRXN1* exon numbering used in this P379 NRXN1 product description is the exon numbering derived from MANE project based on MANE Select transcript NM_001330078.2. Two probes are included in this probemix for an alternative exon present in the beta transcript variant NM_138735.4. The *NRXN1* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version 3 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select NM_ sequence for this gene. 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

P379 NRXN1 contains 43 MLPA probes with amplification products between 130 and 449 nucleotides (nt). This includes 32 probes for the *NRXN1*, of which, two probes are for an alternative exon present in the beta transcript variant NM_138735.4. In addition, 11 reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

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

MLPA technique

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

MLPA technique validation

Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from 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 (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 deviations to the indicated copy number alteration (CNA) findings might occur.

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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/gain	$1.30 < FR < 1.65$
Heterozygous triplication/homozygous duplication/gain	$1.75 < FR < 2.15$
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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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 *NRXN1* gene are small (point) mutations, none of which will be detected by using 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. 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 in sequence data 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.

NRXN1 mutation database

<https://databases.lovd.nl/shared/genes/NRXN1>. 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 <https://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 *NRXN1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. P379-A2 NRXN1

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

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

« 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.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

Table 2. NRXN1 probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene exon ^a	Ligation site ^b NM_001330078.2	Partial sequence ^c (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	<i>1074-1076 (exon 2)</i>		
409	15891-L17984	Upstream (Exon 1)	269 nt before exon 1	ATGCAAAGGTAA-AAGCACCTCGCT	0.3 kb
135	15861-L17954	Exon 1	59-60	TCTGAAAAGGCA-GCAGCAGTCGCC	3.3 kb
185	16657-L19735	Exon 2	565-566	AGCATCCAAGGA-TACTGGCTCACT	2.2 kb
270 Ø	16659-L19210	Intron 2 (Exon 3)	961 nt after exon 2	TAATTTCTGTGG-TTCTTGGGGCTT	100.6 kb
228 Ж	15873-SP0298-L17966	Exon 3 (4)	25 nt before exon 3, 1849-1850	TTTTCTTTTCT-29 nt spanning oligo-CAACAATGTGGA	3.2 kb
418	15892-L17985	Exon 4 (5)	27 nt before exon 4	CCTTGTTTATTG-CTGACTGTGCTC	291.5 kb
166 Ø	15865-L18430	Intron 5 (Exon 6)	7.6 kb before exon 6	GAACAAGAGAAG-TTAGACTCAGAA	7.7 kb
306	15881-L18439	Exon 6 (7)	2051-2052	CACACTGGGAAA-TCGGCTGATTAT	2.2 kb
277 #	20622-L18437	Exon 7 (8)	2219-2218 reverse	ATAGCGTGTCCA-ATGCCTGAGTGC	1.1 kb
215	15872-L17965	Exon 8 (9)	2 nt before exon 8	GCTTTGTTATCT-AGGTGACAATAT	67.2 kb
148	15863-L17956	Exon 9 (10)	2445-2446	GACTTGCCAAGC-AAGGAGATCCTA	0.3 kb
400	15890-L17983	Exon 9 (10)	2792-2793	GTGAATGATGGA-GAATGGTATCAT	14.0 kb
364	15887-L17980	Exon 10 (11)	2866-2867	GCGTACTCCCTA-CACTGCTCCTGG	7.3 kb
178	15867-L17960	Exon 11 (12)	3333-3334	GTGCATATGGCA-TTCTGATGGCAA	2.6 kb
142	16305-L18830	Exon 12 (13)	69 nt before exon 12	TGGACTGTAATT-TTATTGGATGTC	22.1 kb
263	15878-L18436	Exon 13 (14)	3482-3483	GCTGGCTATAAC-CTCAATGATAAC	8.9 kb
247	15876-L18435	Exon 14 (15)	3635-3636	ATCATCACAGAA-CGACGGTATCTT	1.7 kb
439	16188-L28435	Exon 15 (16)	4076-4077	AGCAACCTCCAC-ACTGTAAAGATT	23.6 kb
427	15893-L17986	Exon 16 (17)	4223-4224	GAAGGCTTTCAA-GGCTGCCTGGCA	6.9 kb
346	15885-L17978	Exon 17 (18)	4364-4365	AATCAAGGTGTG-TGCTTGCAACAA	117.8 kb
196 « Ø	15869-L18432	Intron 17	111 kb before exon 18; 121-122 (NM_138735.5)	TTCTTCTCAGCT-GCCTTTCCGCCG	1.0 kb
256 « Ø	15877-L17970	Intron 17	110 kb before exon 18; 1080-1081 (NM_138735.5)	GTGCCTATTGCA-ATCTACAGGTCA	109.8 kb
233	15874-L18434	Exon 18 (19)	4448-4449	GCTGGGACGACA-TATATCTTTAGC	0.1 kb
286	15880-L18438	Exon 18 (19)	4584-4585	GAGTGGACAGTT-CTTCAGGCTTGG	145.4 kb
336	15886-L18441	Exon 19 (20)	4655-4656	TTTAATGTTGGG-ACAGATGACATC	0.1 kb
312	15882-L18440	Exon 19 (20)	4727-4728	GTTCGTTTCACG-AGGAGTGGTGGC	36.4 kb
157	15864-L18520	Exon 20 (21)	4838-4839	CAGCGAATTCCA-TATCGACTTGGT	1.5 kb
373	15888-L17981	Exon 21 (22)	4994-4995	GGCTTGAAAGTT-CTGAATATGGCA	109.7 kb
172	15866-L17959	Exon 22 (23)	5209-5210	GCAGACCACAGA-TGACATCCTTGT	0.1 kb
299	15883-L17976	Exon 22 (23)	53 nt after exon 22	ATCTTTGTTGCA-TGTTATATTCCT	21.5 kb
208	15871-L18433	Exon 23 (24)	5441-5442	TACAAGTACAGA-AACCGGGATGAA	0.6 kb
391	15889-L17982	Exon 23 (24)	6032-6033	AGTCAGTCCAAT-TGCCAGAGAAGA	
		<i>stop codon</i>	<i>5595-5597 (exon 23)</i>		

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

^b Ligation sites are relative to the start of the NM_L sequence, and not relative to the coding sequence.

^c Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« 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.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

Related products

For related products, see the [product page](#) on our website.

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 A2-03 – 12 October 2026 (05P) - Product description rewritten and adapted to a new template. - Adjusted exon numbering according to the MANE select transcript in Table 1 and 2. Version A2-02 – 11 December 2024 (01P) - The term 'mental retardation', here used to further explain 'intellectual disability', is considered outdated and was removed. 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.

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