Product Description SALSA® MLPA® Probemix P254-B2 PSEN1

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, three reference probes have been replaced and one probe length has been adjusted. For complete product history see page 5.

Catalogue numbers:
- **P254-025R:** SALSA MLPA Probemix P254 PSEN1, 25 reactions.
- **P254-050R:** SALSA MLPA Probemix P254 PSEN1, 50 reactions.
- **P254-100R:** SALSA MLPA Probemix P254 PSEN1, 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 P254 PSEN1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PSEN1* gene, which is associated with Alzheimer's Disease (AD).

AD patients with an inherited form of the disease mostly carry mutations in the presenilin proteins (PSEN1; PSEN2) or in the amyloid precursor protein (APP) which is made in the brain and other tissues. These disease-linked mutations result in increased production of the longer form of amyloid-beta (main component of amyloid deposits found in AD brains). Presenilins are postulated to regulate APP processing through their effects on gamma-secretase, an enzyme that cleaves APP. Furthermore, it is believed that the presenilins are involved in cleavage of the Notch receptor, either via the gamma-secretase complex or via direct protease activity.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1236/.

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 P254-B2 PSEN1 contains 27 MLPA probes with amplification products between 136 and 463 nt. This includes 14 probes for the *PSEN1* gene, with one probe for each of the 12 exons and two additional probes targeting exon 1 and intron 9. In addition, 13 reference probes are included and detect 13 different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity Fragments (Q-fragments), two DNA Denaturation Fragments (D-fragments), one benchmark fragment, 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 105 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 105 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 Alzheimer's disease. 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 *PSEN1* gene are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemix P254 PSEN1.
- 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.

***PSEN1* mutation database:** http://www.molgen.ua.ac.be/ADmutations/. We strongly encourage users to deposit positive results in the Alzheimer Disease & Frontotemporal Dementia Mutation Database (AD&FTDMDB). 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 *PSEN1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P254-B2 PSEN1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*PSEN1*
64-105	Control fragments – see table in probemix content section for more information		
136 *	Reference probe 09938-L10397	8q13	
142	**PSEN1 probe** 07507-L07169		**Exon 1**
160	**PSEN1 probe** 07508-L07170		**Exon 1**
166	Reference probe 04825-L04209	5p13	
178	**PSEN1 probe** 07509-L07171		**Exon 2**
184	Reference probe 14413-L16118	10q26	
196	**PSEN1 probe** 07510-L07172		**Exon 3**
202	Reference probe 07820-L07574	1q31	
214 «	**PSEN1 probe** 08447-L07174		**Exon 5**
229	Reference probe 01828-L01393	16p13	
238	**PSEN1 probe** 08446-L07173		**Exon 4**
257	Reference probe 01055-L00628	17q21	
269 ¥ «	**PSEN1 probe** 07513-L31299		**Exon 6**
282	Reference probe 05387-L04784	12p11	
292 «	**PSEN1 probe** 17510-L21355		**Exon 7**
310	Reference probe 03934-L03389	15q21	
318	**PSEN1 probe** 07515-L07177		**Exon 8**
337	Reference probe 03264-L02701	3q29	
346	**PSEN1 probe** 07516-L07178		**Exon 9**
373	**PSEN1 probe** 07517-L07179		**Intron 9**
391	Reference probe 05423-L04833	6p24	
399	**PSEN1 probe** 07518-L07180		**Exon 10**
409	Reference probe 02718-L00732	14q11	
425	**PSEN1 probe** 07519-L07181		**Exon 11**
445 *	Reference probe 06367-L05883	18p11	
454	**PSEN1 probe** 07520-L07182		**Exon 12**
463 *	Reference probe 09038-L09292	2q37	

* New in version B2 (from lot B2-0718 onwards).

¥ Changed in version B2 (from lot B2-0718 onwards). Small change in length, no change in sequence detected.

« 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.

Note: The exon numbering used in this P254-B2 PSEN1 product description is the exon numbering from the RefSeq transcript NM_000021.3, which is identical to the NG_007386.2 sequence. The exon numbering and NM sequence used is from 07/2018, 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. PSEN1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	PSEN1 exon	Ligation site NM_000021.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*285-287 (Exon 3)*		
142	07507-L07169	Exon 1	81-82	ATCGGAAACAAA-ACAGCGGCTGGT	0.3 kb
160	07508-L07170	Exon 1	255 nt after exon 1	TTTGCTCGAAGA-CGTCTCAGGGCG	11.0 kb
178	07509-L07171	Exon 2	176-177	CTGCAAGTGACA-ACAGCCTTTGCG	0.2 kb
196	07510-L07172	Exon 3	322-323	CTACTTCCAGAA-TGCACAGATGTC	22.8 kb
238	08446-L07173	Exon 4	434-435	CCTGAGCCATTA-TCTAATGGACGA	2.8 kb
214 «	08447-L07174	Exon 5	750-751	TTCTGTATAAAT-ACAGGTGCTATA	13.1 kb
269 «	07513-L31299	Exon 6	15 nt before exon 6	ATTATATTGAAA-TGCTTTCTTTTC	6.0 kb
292 «	17510-L21355	Exon 7	1041-1042	TCTTGGCTGTGA-TTTCAGTATATG	5.2 kb
318	07515-L07177	Exon 8	1089-1090	AAGGTCCACTTC-GTATGCTGGTTG	8.3 kb
346	07516-L07178	Exon 9	1175-1176	TGGTTGGTGAAT-ATGGCAGAAGGA	2.5 kb
373 #	07517-L07179	Intron 9	2391 nt after exon 9	CCTGCTGTCTAT-AGCTCCCATGGC	2.9 kb
399	07518-L07180	Exon 10	1276-1277	TGTTGCAGAGAA-TGATGATGGCGG	5.4 kb
425	07519-L07181	Exon 11	1487-1488	GCAACAGCCAGT-GGAGACTGGAAC	2.8 kb
454	07520-L07182	Exon 12	2378-2379	ATTGCCATTTCT-TCCCAAGGCCAG	
		stop codon	*1686-1688 (Exon 12)*		

« 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.
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 P254-B2 PSEN1 product description is the exon numbering from the RefSeq transcript NM_000021.3, which is identical to the NG_007386.2 sequence. The exon numbering and NM sequence used is from 07/2018, 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

P170 APP Contains probes for the *APP* gene, involved in early-onset Alzheimer's disease (ADEOAD).

P471 EOFAD Contains probes for all exons of the *PSEN1*, *PSEN2*, and *APP* genes, involved in early-onset familial Alzheimer's disease (EOFAD).

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P254 PSEN1

- Lindquist S et al. (2009). Genetic testing in familial AD and FTD: mutation and phenotype spectrum in a Danish cohort. *Clin. Genet.* 76(2), 205-209.

P254 Product history	
Version	*Modification*
B2	Three reference probes have been replaced and one probe length has been adjusted.
B1	The *PSEN1* exon 7 probe and six reference probes have been replaced. The 88, 96, 100 and 105 nt control fragments (QDX2) have been included.
A1	First release.

Implemented changes in the product description
Version B2-01 – 31 August 2018 (01P) - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. *Version 08 – 04 December 2015 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Exon numbering adjusted according to RefSeq sequence. *Version 07 (48)* - Electropherogram picture of old buffer (introduced December 2012) removed. *Version 06 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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