Product Description SALSA® MLPA® Probemix P471-A1 EOFAD

To be used with the MLPA General Protocol.

Version A1. For complete product history see page 7.

Catalogue numbers:
- **P471-025R:** SALSA MLPA Probemix P471 EOFAD, 25 reactions.
- **P471-050R:** SALSA MLPA Probemix P471 EOFAD, 50 reactions.
- **P471-100R:** SALSA MLPA Probemix P471 EOFAD, 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 P471 EOFAD is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PSEN1*, *PSEN2*, and *APP* genes, which are associated with Early-Onset Familial Alzheimer Disease (EOFAD).

Alzheimer disease (AD) is a chronic neurodegenerative disorder characterized by adult-onset progressive dementia associated with cerebral cortical atrophy, beta-amyloid plaque formation, and intraneuronal neurofibrillary tangles. Patients usually suffer from failure of memory, and other features including poor judgement, confusion and language disturbance. AD can be diagnosed in families if multiple members are affected (familial AD; FAD). Early-onset FAD (EOFAD) refers to families in which onset is consistently before the age of 60 to 65 years and often before the age of 55 years.

EOFAD has a genetic component and is inherited in an autosomal dominant manner. Defects in the *PSEN1* gene on chromosome 14, *APP* gene on chromosome 21, and *PSEN2* gene on chromosome 1 are the main cause of EOFAD. The protein encoded by the *APP* gene is the amyloid beta precursor protein which is a cell surface receptor and transmembrane precursor protein that is cleaved by secretases to form a number of peptides. A part of these peptides form the protein basis of the amyloid plaques found in the brains of patients with AD. The proteins encoded by the *PSEN1* and *PSEN2* genes are presenilin proteins 1 and 2, respectively. Presenilins are thought to be involved in regulation of the cleavage of the amyloid beta precursor protein (APP) and of the Notch receptor.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1161/

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 *PSEN1*, *APP*, and *PSEN2* exon numberings used in this P471-A1 EOFAD product description are the exon numberings from the NG_007386.2 sequence, the NG_007376.1 sequence, and the NG_007381.1 sequence, respectively. The exon numbering and NM sequence used is from 05/2019 but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Probemix content: The SALSA MLPA Probemix P471-A1 EOFAD contains 53 MLPA probes with amplification products between 124 and 504 nucleotides (nt). This includes 12 probes for the *PSEN1* gene, one probe or each exon, 20 probes for the *APP* gene, one probe for each exon and an additional probe upstream of exon 1 and for intron 1, and 13 probes for *PSEN2* gene, one probe for each exon. In addition, eight 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.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 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 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 Early-Onset Familial Alzheimer 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 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 for autosomal chromosomes or pseudo-autosomal regions:

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 (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 *PSEN1*, *PSEN2*, and *APP* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P471 EOFAD.
- 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.

Alzheimer Disease & Frontotemporal Dementia mutation database: http://www.molgen.ua.ac.be/Admutations/. We strongly encourage users to deposit positive results in the 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 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P471-A1 EOFAD

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*APP*	*PSEN1*	*PSEN2*
64-105	Control fragments – see table in probemix content section for more information				
124	Reference probe 02855-L27817	18q21			
130	**PSEN2 probe** 20834-L28852				**Exon 9**
136	**APP probe** 20835-L28853		**Exon 4**		
141	Reference probe 03088-L22752	16p13			
148 «	**PSEN1 probe** 20836-L28854			**Exon 6**	
154	**PSEN2 probe** 20837-L28855				**Exon 8**
160	**PSEN1 probe** 20838-L28856			**Exon 9**	
166	**APP probe** 20839-L28983		**Upstream**		
171	**PSEN2 probe** 20840-L28858				**Exon 10**
179	Reference probe 17403-L21112	3p21			
184	**PSEN1 probe** 20841-L28859			**Exon 10**	
190	**PSEN2 probe** 20842-L28860				**Exon 7**
196	**APP probe** 20843-L28861		**Intron 1**		
202	**PSEN2 probe** 20844-L28862				**Exon 6**
207	**APP probe** 20845-L28863		**Exon 12**		
213	**PSEN1 probe** 20846-L28864			**Exon 11**	
220	**PSEN2 probe** 20847-L28865				**Exon 13**
226	**APP probe** 20848-L28866		**Exon 10**		
232	**PSEN2 probe** 20849-L28867				**Exon 4**
237	**APP probe** 20850-L28868		**Exon 18**		
241 «	**APP probe** 20851-L28869		**Exon 1**		
250	**APP probe** 20852-L28870		**Exon 15**		
257	Reference probe 14738-L16435	4q22			
263	**APP probe** 20853-L28871		**Exon 7**		
268 «	**PSEN2 probe** 20854-L28997				**Exon 1**
274	**APP probe** 20855-L28873		**Exon 14**		
281 «	**PSEN1 probe** 20856-L28874			**Exon 7**	
286	**APP probe** 20857-L28985		**Exon 6**		
292	**PSEN2 probe** 20858-L28876				**Exon 11**
299	**PSEN1 probe** 20859-L28996			**Exon 5**	
304	**APP probe** 20860-L28878		**Exon 9**		
310	**PSEN1 probe** 20861-L28879			**Exon 4**	
319	**APP probe** 20862-L28880		**Exon 11**		
328	**APP probe** 20863-L28881		**Exon 2**		
337	**PSEN1 probe** 20864-L28882			**Exon 3**	
346	Reference probe 15885-L17978	2p16			
355	**APP probe** 20865-L28883		**Exon 5**		
364	**PSEN1 probe** 20866-L28884			**Exon 2**	
373	**APP probe** 20867-L28885		**Exon 13**		
382	**PSEN2 probe** 20868-L28886				**Exon 12**
391	**APP probe** 20869-L28887		**Exon 3**		
401	**PSEN1 probe** 20870-L28888			**Exon 12**	
409	**PSEN1 probe** 20871-L28889			**Exon 1**	
418	Reference probe 14382-L11818	13q22			
427	**PSEN2 probe** 20872-L28890				**Exon 5**
436	**APP probe** 20873-L28891		**Exon 8**		
445	Reference probe 08276-L08155	8q23			
454	**APP probe** 20874-L28892		**Exon 17**		
463 «	**PSEN2 probe** 20875-L28893				**Exon 3**
472	**APP probe** 20876-L28894		**Exon 16**		
483 «	**PSEN2 probe** 20877-L28895				**Exon 2**
495	**PSEN1 probe** 20878-L28896			**Exon 8**	
504	Reference probe 13438-L24633	5q31			

a) See above section on exon numbering 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.

Table 2. P471-A1 probes arranged according to chromosomal location

Table 2a. *PSEN1* gene

Length (nt)	SALSA MLPA probe	*PSEN1* exon[a]	Ligation site NM_000021.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*213-215 (Exon 3)*		
409	20871-L28889	Exon 1	5 nt before exon 1	GTCCGCGGTTTC-ACATCGGAAACA	11.4 kb
364	20866-L28884	Exon 2	142-143	TGGCCTGGAGGA-GAACACATGAAA	0.1 kb
337	20864-L28882	Exon 3	189-190	TTTCTGTGAAAC-AGTATTTCTATA	22.8 kb
310	20861-L28879	Exon 4	2 nt before exon 4	TCTTGTGCTTAT-AGAATGACAATA	2.8 kb
299	20859-L28996	Exon 5	4 nt before exon 5	GTTTGTTTTATT-GTAGAATCTATA	13.4 kb
148 «	20836-L28854	Exon 6	60 nt after exon 6 reverse	GGCTTTAAATGA-TAGCTACACAGC	5.7 kb
281 «	20856-L28874	Exon 7	816-817	TTGCACTCCTGA-TCTGGAATTTTG	5.3 kb
495	20878-L28896	Exon 8	63 nt before exon 8	ATTCCTCCCTAC-CACCCATTTACA	8.4 kb
160	20838-L28856	Exon 9	1091-1092	GCAACAATGGTG-TGGTTGGTGAAT	5.4 kb
184	20841-L28859	Exon 10	1176-1177	AAGGCACAGAAA-GGGAGTCACAAG	5.5 kb
213	20846-L28864	Exon 11	63 nt after exon 11 reverse	ACTGCCTTAAAG-GGACTGTGTAAT	2.1 kb
401	20870-L28888	Exon 12	1709-1710	TCCACATCTAAC-AAAGTCAAGATT	
		stop codon	*1614-1616 (Exon 12)*		

Table 2b. *APP* gene

Length (nt)	SALSA MLPA probe	*APP* exon[a]	Ligation site NM_000484.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*151-153 (Exon 1)*		
166 +	20839-L28983	Upstream	NM_001136131.2; 80-79 reverse	CAGGATCAGGGA-AAGGTGAGTCCT	
241 «	20851-L28869	Exon 1	13-12 reverse	CCTACCGCTGCC-GAGGAAACTGAC	0.3 kb
196 +	20843-L28861	Intron 1	NM_001136016.3; 172-173	TGACAATGATTG-GAGCCAGCTCTT	30.6 kb
328	20863-L28881	Exon 2	301-302	TCCAGAATGGGA-AGTGGGATTCAG	28.2 kb
391	20869-L28887	Exon 3	489-490	CACTTTGTGATT-CCCTACCGCTGC	22.1 kb
136	20835-L28853	Exon 4	562-563	AATTCTTACACC-AGGAGAGGATGG	36.7 kb
355	20865-L28883	Exon 5	777-778	TCGGATGTCTGG-TGGGGCGGAGCA	2.3 kb
286	20857-L28985	Exon 6	948-949	GAAGAAGCCACA-GAGAGAACCACC	29.1 kb
263	20853-L28871	Exon 7	1098-1099	GAAGGGAAGTGT-GCCCCATTCTTT	21.8 kb
436	20873-L28891	Exon 8	15 nt after exon 8	CGTTGTCATTCA-CCTGAGGGAAGG	2.8 kb
304	20860-L28878	Exon 9	1263-1264	GCAGCCAGTACC-CCTGATGCCGTT	14.9 kb
226	20848-L28866	Exon 10	1422-1421 reverse	TTATCAGCTTTA-GGCAAGTTCTTT	6.5 kb
319	20862-L28880	Exon 11	1594-1595	CCGCTCTGCAGG-CTGTTCCTCCTC	0.9 kb
207	20845-L28863	Exon 12	1694-1695	TTTCGAGCATGT-GCGCATGGTGGA	19.4 kb
373	20867-L28885	Exon 13	1747-1748	AGGTTATGACAC-ACCTCCGTGTGA	1.0 kb
274	20855-L28873	Exon 14	1911-1910 reverse	TCGTTTCCGTAA-CTGATCCTTGGT	42.8 kb
250	20852-L28870	Exon 15	2069-2068 reverse	GGCGGGCATCAA-CAGGCTCAACTG	6.8 kb
472	20876-L28894	Exon 16	61 nt after exon 16 reverse	CAGGATGAACCA-GAGTTAATAGGT	7.6 kb
454	20874-L28892	Exon 17	2351-2352	CATTCATCATGG-TGTGGTGGAGGT	5.8 kb
237	20850-L28868	Exon 18	2424-2425	AACGGCTACGAA-AATCCAACCTAC	10.0 kb
		stop codon	*2461-2463 (Exon 18)*		

Table 2c. *PSEN2* gene

Length (nt)	SALSA MLPA probe	*PSEN2* exon[a]	Ligation site NM_000447.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*384-386 (Exon 4)*		
268 «	20854-L28997	Exon 1	11-12	TGCGTAAACTCC-GCTGGAGCGCGG	0.6 kb
483 «	20877-L28895	Exon 2	11 nt before exon 2	GCGGTGTTTGGC-TGTTTTATCAGG	4.2 kb
463 «	20875-L28893	Exon 3	273-272 reverse	TCCCTGGCTTTC-AAAGAGGGCAGC	6.4 kb
232	20849-L28867	Exon 4	365-366	CTTTTTCCAGGT-GCTTCCAGAGGC	1.9 kb
427	20872-L28890	Exon 5	626-627	GAGCTGACCCTC-AAATACGGAGCG	1.8 kb
202	20844-L28862	Exon 6	857-856 reverse	TACTTGTAGAGC-ACCACCAAGAAG	2.5 kb
190	20842-L28860	Exon 7	7 nt after exon 7	CTTGGGTAAGTG-ACAGATAAGCAG	0.7 kb
154	20837-L28855	Exon 8	5 nt before exon 8 reverse	CACTTCCCTGCA-GGACCAAGGTGG	1.3 kb
130	20834-L28852	Exon 9	1217-1216 reverse	TGGGCAGTTTCT-ACCAGCATTCTC	1.2 kb
171	20840-L28858	Exon 10	1275-1274 reverse	CGTCCACACCAT-GGCAGCTGGGGG	0.5 kb
292	20858-L28876	Exon 11	1416-1417	AGCCTCCCTTGA-CTGGCTACCCAG	2.2 kb
382	20868-L28886	Exon 12	1462-1463	CACAGGGGGCGT-GAAGCTTGGCCT	1.6 kb
220	20847-L28865	Exon 13	1723-1722 reverse	TCCCTCAGATGT-AGAGCTGATGGG	
		stop codon	*1728-1730 (Exon 13)*		

a) See 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.

« 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.
+ The 20839-L28983 probe is located in exon 1 of the NM_001136131.2 transcript variant and the 20843-L28861 probe is located in exon 1 of the NM_001136016.3 transcript variant, which are both not present in NM_000484.

Related SALSA MLPA probemixes

P170 APP	Contains probes for the APP gene. Probes present in this probemix have a different ligation site than those in P471.
P254 PSEN1	Contains probes for the PSEN1 gene. Probes present in this probemix have a different ligation site than those in P471.
P275 MAPT-GRN	Contains probes for the MAPT and GRN genes, implicated in Alzheimer's disease/dementia.

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.

P471 Product history	
Version	*Modification*
A1	First release

Implemented changes in the product description
Version A1-01 — 27 September 2019 (02P) - Product description rewritten and adapted to a new template. - Warning about probes sensitive to salt contamination added in Tables. - Ligation sites of the probes targeting the *PSEN1*, *PSEN2,* and *APP* genes updated according to new versions of the NM_ reference sequences. *Version 02 – 15 February 2017 (55)* - Warning removed in Table 1 and 2, 268 nt probe 20854-L28997 and 483 nt probe 20877-L28895. - Information about the number of reference probes added on page 1. - Various minor textual and layout changes. *Version 01 – 04 March 2016 (55)* - Not applicable, new document.

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