Product Description SALSA® MLPA® Probemix P490-B1 ADA2

To be used with the MLPA General Protocol.

Version B1.

Catalogue numbers:
- **P490-025R:** SALSA MLPA Probemix P490 ADA2, 25 reactions.
- **P490-050R:** SALSA MLPA Probemix P490 ADA2, 50 reactions.
- **P490-100R:** SALSA MLPA Probemix P490 ADA2, 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 P490 ADA2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *ADA2* gene, which is associated with Deficiency of Adenosine Deaminase 2 (DADA2).

DADA2 is a rare autosomal recessive genetic disease, caused by defects in the adenosine deaminase 2 (*ADA2*, also known as *CECR1*) gene. Symptoms include systemic inflammation, vasculitis, early-onset ischemic strokes and immune deficiency. ADA2 controls the level of extracellular adenosine and thereby signalling through adenosine receptors. ADA2 influences differentiation of macrophages and is thought to have growth factor activity (Meyts and Aksentijevich 2018).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK544951/.

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 *ADA2* exon numbering used in this P490-B1 ADA2 product description is the exon numbering from the RefSeq transcript NM_001282227.2 (transcript variant 5). The exon numbering and NM_ sequence used have been retrieved on 03/2020. 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 P490-B1 ADA2 contains 22 MLPA probes with amplification products between 151 and 346 nucleotides (nt). This includes 12 probes for the *ADA2* gene, covering all exons of the gene. In addition, ten 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, 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 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 DADA2. 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. Sample ID numbers NA02944 and NA16362 from the Coriell Institute have been tested with this P490-B1 probemix at MRC-Holland and can be used as positive control samples to detect a heterozygous deletion and a heterozygous duplication of the *ADA2* gene, respectively. 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 0software 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

Copy number status	Dosage quotient
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 *ADA2* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P490 ADA2.
- 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.

***ADA2* mutation database:** https://databases.lovd.nl/shared/genes/CECR1. 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 *ADA2* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P490-B1 ADA2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	ADA2
64-105	Control fragments – see table in probemix content section for more information		
151	Reference probe 21049-L29278	10q24	
158	**ADA2 probe** 22435-L31611		**Exon 7**
172	**ADA2 probe** 22432-L30088		**Intron 4**
179	Reference probe 17403-L21112	3p21	
184	**ADA2 probe** 22429-L30090		**Exon 2**
192	Reference probe 14964-L16700	6q22	
196	**ADA2 probe** 22437-L31612		**Exon 9**
208	**ADA2 probe** 22430-L31610		**Exon 3**
220	Reference probe 17867-L22126	19q13	
229	**ADA2 probe** 22433-L30042		**Exon 5**
238	Reference probe 19807-L14372	5p15	
244	**ADA2 probe** 22428-L30123		**Intron 1**
253	Reference probe 16399-L28036	17q22	
263	**ADA2 probe** 22434-L30040		**Exon 6**
271	Reference probe 20728-L05861	1p21	
280	**ADA2 probe** 22438-L30036		**Exon 10**
289	Reference probe 22614-L31904	7q31	
299	**ADA2 probe** 22427-L30124		**Exon 1**
317	**ADA2 probe** 22431-L30089		**Exon 4**
328	Reference probe 19605-L29238	8q13	
337	**ADA2 probe** 22436-L30035		**Exon 8**
346	Reference probe 14302-L15972	15q13	

a) See above section on exon numbering for more information.

Table 2. ADA2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	ADA2 exon[a]	Ligation site NM_001282227.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*170-172 (Exon 2)*		
299	22427-L30124	Exon 1	86-87	CCAGGATCTAAC-TGGGCTCTGATT	2.3 kb
244 ◍	22428-L30123	Intron 1	2.2 kb after exon 1 *reverse*	GGGATCCAACTA-GAGGCCGGGGAG	10.0 kb
184	22429-L30090	Exon 2	267-266 *reverse*	TCAGGGTCCTCA-TGGCCTCCTTCA	2.3 kb
208	22430-L31610	Exon 3	526-527	AAATGTTCCAAG-TGGATTCTGCTG	3.4 kb
317	22431-L30089	Exon 4	643-644	TACACAAACCAA-AATGTTGTCTGG	4.1 kb
172 +	22432-L30088	Intron 4	4.0 kb after exon 4 *reverse*	CCCCTCCGGAAA-AAGGAAAATTGA	7.8 kb
229	22433-L30042	Exon 5	841-840 *reverse*	TGGTAAGTCTTC-ACTGACCACTCT	1.8 kb
263	22434-L30040	Exon 6	951-952	TGTCATCGCAGA-ATCCATCCGAAT	1.6 kb
158	22435-L31611	Exon 7	1038-1039	GGACACTGGCCA-CTCCTTGCATGA	5.7 kb
337	22436-L30035	Exon 8	1205-1206	ATGGATTTGCTT-TGAGCAAACACC	0.9 kb
196	22437-L31612	Exon 9	1477-1478	CTGGCCATGAAC-TCTATCAAGTGA	0.3 kb
280	22438-L30036	Exon 10	1561-1562	AAGTTCATAGCA-GATGTGGCTACA	
		stop codon	*1577-1579 (Exon 10)*		

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.

ⓐ This probe targets an exon in transcript variant 7, NM_001282229.1 (ligation site 91-90 *reverse*).
+ This probe targets an exon in transcript variant 2, NM_177405.3 (ligation site 64-63 *reverse*).

References

- Meyts I and Aksentijevich I (2018). Deficiency of adenosine deaminase 2 (DADA2): updates on the phenotype, genetics, pathogenesis, and treatment. *J Clin Immunol*. 38(5):569–578.
- 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 P490 ADA2

- Lee PY et al. (2018). Disrupted N-linked glycosylation as a disease mechanism in deficiency of ADA2. *J Allergy Clin Immunol.* 142(4): 1363–1365.e8.

P490 Product history	
Version	*Modification*
B1	First commercial release.

Implemented changes in the product description
Version B1-01 — 29 April 2020 (02P) - Not applicable, new document.

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