Product Description
SALSA® MLPA® Probemix P388-A2 AGS

To be used with the MLPA General Protocol.

Version A2
For complete product history see page 8.

Catalogue numbers:
- **P388-025R:** SALSA MLPA Probemix P388 AGS, 25 reactions.
- **P388-050R:** SALSA MLPA Probemix P388 AGS, 50 reactions.
- **P388-100R:** SALSA MLPA Probemix P388 AGS, 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.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, 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
The SALSA MLPA Probemix P388 AGS is a **research use only (RUO)** assay for the detection of deletions or duplications in the *TREX1*, *RNASEH2B*, *RNASEH2C*, *RNASEH2A,* and *SAMHD1* genes, which are associated with Aicardi-Goutières Syndrome (AGS).

AGS is a genetically determined encephalopathy, characterised by calcification of the basal ganglia and white matter, demyelination and raised levels of lymphocytes in the cerebrospinal fluid. AGS is a heterogeneous disorder. To date mutations in several genes are linked to AGS, including *TREX1*, *RNASEH2B*, *RNASEH2C*, *RNASEH2A*, and *SAMHD1*. These are known as AGS1, AGS2, AGS3, AGS4 and AGS5, respectively. More recently, two additional genes (*ADAR1* and *IFIH1*) have been described in AGS patients. Probes for these genes are not present in this probemix.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1475/.

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 *TREX1*, *RNASEH2B*, *RNASEH2C*, *RNASEH2A,* and *SAMHD1* exon numbering used in this P388-A2 AGS product description is the exon numbering from the LRG_282, LRG_279, LRG_280, LRG_278, and LRG_281 sequences, respectively. The *TREX1* 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 02 onwards, we have adopted the LRG sequence exon numbering. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained

from the LRG sequences. 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
The SALSA MLPA Probemix P388-A2 AGS contains 48 MLPA probes with amplification products between 130 and 504 nucleotides (nt). This includes 40 probes targeting the aforementioned genes. 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.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 the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.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 different unrelated individuals who are from families without a history of AGS. 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 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.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	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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 the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software 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. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) 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 aforementioned genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P388 AGS.
- 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 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.

LOVD mutation database

https://databases.lovd.nl/shared/genes/. 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 SNVs and unusual results (e.g., a duplication of *SAMHD1* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P388-A2 AGS

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*TREX1*	*SAMHD1*	*RNASEH2B*	*RNASEH2C*	*RNASEH2A*
64-105	Control fragments – see table in probemix content section for more information						
130	Reference probe 00797-L13645	5q					
142	**SAMHD1 probe** 16251-L18543			**Exon 10**			
148	**RNASEH2B probe** 16252-L18544				**Exon 5**		
155	**RNASEH2A probe** 16253-L18545						**Exon 3**
160	**SAMHD1 probe** 16254-L19129			**Exon 8**			
167	**RNASEH2B probe** 16255-L19128				**Exon 2**		
172	Reference probe 14464-L16184	4q					
178	**SAMHD1 probe** 16256-L18548			**Exon 2**			
184	**RNASEH2A probe** 16257-L18549						**Exon 7**
190	**RNASEH2C probe** 16258-L18550					**Exon 2**	
196	**TREX1 probe** 16259-L18551		**Exon 2**				
202	**RNASEH2B probe** 16260-L18552				**Exon 8**		
208	**SAMHD1 probe** 16261-L18553			**Exon 9**			
214	Reference probe 13265-L15166	1p					
220	**RNASEH2A probe** 16262-L18554						**Exon 8**
226 Ж	**RNASEH2A probe** 17002-SP0338-L18583						**Exon 2**
232	**RNASEH2B probe** 16264-L18556				**Exon 10**		
238	**RNASEH2A probe** 16265-L18557						**Exon 4**
244	**SAMHD1 probe** 16266-L19130			**Exon 13**			
250	**SAMHD1 probe** 16267-L18559			**Exon 3**			
262 Ж	**SAMHD1 probe** 16268-SP0335-L18560			**Exon 7**			
269	**RNASEH2A probe** 16269-L19283						**Exon 1**
274	**SAMHD1 probe** 16270-L18562			**Exon 11**			
283	**RNASEH2B probe** 16271-L18563				**Exon 3**		
292 Ж	**RNASEH2C probe** 16272-SP0336-L18564					**Exon 3**	
301	**RNASEH2B probe** 16273-L18565				**Exon 7**		
310	Reference probe 10689-L11271	6p					
319	**SAMHD1 probe** 16274-L18566			**Exon 12**			
326	**RNASEH2A probe** 16275-L18567						**Exon 5**
337	**RNASEH2B probe** 16276-L18568				**Exon 1**		
346	**SAMHD1 probe** 16277-L18569			**Exon 15**			
355	**RNASEH2B probe** 16278-L19474				**Exon 4**		
364	**SAMHD1 probe** 16279-L18571			**Exon 5**			
373	Reference probe 08878-L08934	2p					
382 Ж	**RNASEH2B probe** 16280-SP0337-L18572				**Exon 6**		
390	**SAMHD1 probe** 16281-L18573			**Exon 14**			
400	**RNASEH2C probe** 16282-L18574					**Exon 1**	
409	**RNASEH2B probe** 16283-L18575				**Exon 9**		
418	**RNASEH2C probe** 16284-L18576					**Exon 4**	
427	**SAMHD1 probe** 17000-L18555			**Exon 6**			
436	Reference probe 10634-L11182	8q					
445	**RNASEH2B probe** 16286-L18578				**Exon 11**		
453	**SAMHD1 probe** 16287-L18579			**Exon 1**			
463	Reference probe 14308-L15978	15q					
472	**TREX1 probe** 16289-L18581		**Exon 2**				
481	**SAMHD1 probe** 16290-L18582			**Exon 4**			
492	**SAMHD1 probe** 17001-L18577			**Exon 16**			
504	Reference probe 18539-L23848	17q					

[a] See section Exon numbering on page 1 for more information.

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. When this occurs in reference samples, it can look like an increased signal in the test samples.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P388-A2 probes arranged according to chromosomal location

Table 2a. *TREX1* (AGS1)

Length (nt)	SALSA MLPA probe	*TREX1* exon[a]	Ligation site NM_033629.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*107-109 (Exon 2)*		
472	16289-L18581	Exon 2 (1)	364-365	GAGATCACAGGT-CTGAGCACAGCT	0.3 kb
196	16259-L18551	Exon 2 (1)	665-666	ACACTCGCCTGT-ATGGGCAGTCCC	
		stop codon	*1049-1051 (Exon 2)*		

Table 2b. *RNASEH2B* (AGS2)

Length (nt)	SALSA MLPA probe	*RNASEH2B* exon[a]	Ligation site NM_024570.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*287-289 (Exon 1)*		
337	16276-L18568	Exon 1	132-133	CTCGGAAACGAA-ACGAAATTCGGT	17.4 kb
167	16255-L19128	Exon 2	47 nt before exon 2	AAGGTGAGCAAC-AAAACAGCTGTG	2.3 kb
283	16271-L18563	Exon 3	38 nt after exon 3	TAACTAAACACA-TACTCCAGCTTT	1.1 kb
355	16278-L19474	Exon 4	601-602	ATAAAGGCTGAT-AAGGAGGTGAGT	4.1 kb
148	16252-L18544	Exon 5	16 nt before exon 5	CTTGCTTTCCAA-CTAACTGTTTTT	8.6 kb
382 Ж	16280-SP0337-L18572	Exon 6	104 nt & 146 nt after exon 6	TCTTGTATATTC-42 nt spanning oligo -GAAGGCTTTGAC	2.0 kb
301	16273-L18565	Exon 7	869-870	CAACTGCATTTT-TCTCTGGTGACC	2.6 kb
202	16260-L18552	Exon 8	44 nt after exon 8	ACCATTTGCTTC-TTGGTTTCCCCA	1.4 kb
409	16283-L18575	Exon 9	20 nt after exon 9, reverse	CCCCCAAAGATA-TCTTAGTCACAG	4.4 kb
232	16264-L18556	Exon 10	1043-1044	TAAAGTTATCAG-ATGAGCCTGTAG	2.6 kb
445	16286-L18578	Exon 11	1298-1299	TGACTGTTAATG-ACTACCTTTGGT	
		stop codon	*1223-1225 (Exon 11)*		

Table 2c. *RNASEH2C* (AGS3)

Length (nt)	SALSA MLPA probe	*RNASEH2C* exon[a]	Ligation site NM_032193.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*41-43 (Exon 1)*		
400	16282-L18574	Exon 1	84 nt before exon 1	AAGGGGACTACA-CTTCCCGTGAAG	0.6 kb
190 #	16258-L18550	Exon 2	336-337	GCCAGACCCCTT-GCGGGATTCCGG	0.2 kb
292 Ж	16272-SP0336-L18564	Exon 3	390-391 & 414-415	CCTTCCCCAGGA-24 nt spanning oligo -CTTCAGCCGCTT	0.4 kb
418	16284-L18576	Exon 4	600-601	CTTTGGAACCGA-TTCCATCACCCC	
		stop codon	*533-535 (Exon 4)*		

Table 2d. *RNASEH2A* (AGS4)

Length (nt)	SALSA MLPA probe	*RNASEH2A* exon[a]	Ligation site NM_006397.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*91-93 (Exon 1)*		
269	16269-L19283	Exon 1	45-46	CCGAGACCCGCT-CCTGCAGTATTA	0.4 kb
226 Ж	17002-SP0338-L18583	Exon 2	241-242 & 265-266	ACGCCATCTGTT-24 nt spanning oligo -ATCTGGAGGCGC	0.2 kb
155	16253-L18545	Exon 3	352-353	AGGACACGGACT-TTGTCGGCTGGG	0.2 kb
238	16265-L18557	Exon 4	447-448	CTGTCACATGAT-ACAGCCACTGGG	2.7 kb
326	16275-L18567	Exon 5	590-591	GGTCAAGGCCAA-AGCAGATGCCCT	2.9 kb
	No probe	*Exon 6*			
184	16257-L18549	Exon 7	21 nt before exon 7	CTTGGACTGTCA-CCATTGCCCACC	0.3 kb
220	16262-L18554	Exon 8	897-898	AGGAAGATCACA-TCCTACTTCCTC	
		stop codon	*988-990 (Exon 8)*		

Table 2e. *SAMHD1* (AGS5)

Length (nt)	SALSA MLPA probe	*SAMHD1* exon[a]	Ligation site NM_015474.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*66-68 (Exon 1)*		
453	16287-L18579	Exon 1	195-196	CCGACTACAAGA-CATGGGGTCCGG	4.8 kb
178	16256-L18548	Exon 2	33 nt after exon 2	CACGTTATAACC-AAACACACTTTA	5.6 kb
250	16267-L18559	Exon 3	383-384	TATATCCAGCGA-TTGGTTCAAATC	6.0 kb
481	16290-L18582	Exon 4	487-488	TACACCTCAATT-TCAACGTCTTCG	4.3 kb
364	16279-L18571	Exon 5	650-651	AGTGAACGAGAT-GTTCTCTGTGTT	3.6 kb
427	17000-L18555	Exon 6	722-723	ATGTTTGATGGA-CGATTTATTCCA	7.8 kb
262 Ж	16268-SP0335-L18560	Exon 7	810-811 & 843-844	TTAATTCTAATG-33 nt spanning oligo -TCCCTGAAGAAG	2.4 kb
160	16254-L19129	Exon 8	942-943	GGCGTCCTGAAA-ACAAAAGCTTCC	0.3 kb
208	16261-L18553	Exon 9	1102-1103	AGTAGACAATGA-GTTGCGTATTTG	4.2 kb
142	16251-L18543	Exon 10	1173-1174	GCAACTCTTTAC-ACCGTAGAGCTT	1.3 kb
274	16270-L18562	Exon 11	1315-1316	TGACGACATGGA-AGCCTATACTAA	5.8 kb
319	16274-L18566	Exon 12	1385-1386	AAATTGAAAGAC-GCACGAGAGATT	1.2 kb
244	16266-L19130	Exon 13	1514-1515	GTTGCCAGTGCT-AAACCCAAAGTA	5.8 kb
390	16281-L18573	Exon 14	1654-1655	CAACAGAGCAAT-CAGGATTACTAA	0.5 kb
346	16277-L18569	Exon 15	38 nt before exon 15	CTCCAATGTGTG-ACTTCAAGGTGA	5.6 kb
492	17001-L18577	Exon 16	2525-2526	CCCTGTCACCTC-AAGTTTGAGGAT	
		stop codon	*1944-1946 (Exon 16)*		

[a] See section Exon numbering on page 1 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

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. When this occurs in reference samples, it can look like an increased signal in the test samples.
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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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 P388 AGS

- Crow YJ et al. (2015). Characterization of human disease phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR and IFIH1. *Am J Med Genet A*. 167:296-312.
- Garau J et al. (2019). Molecular genetics and interferon signature in the Italian Aicardi Goutières syndrome cohort: report of 12 new cases and literature review. *J Clin Med*, 8(5), 750.
- Zimmermann M et al. (2018). CRISPR screens identify genomic ribonucleotides as a source of PARP-trapping lesions. *Nature*, 559(7713), 285-289.

P388 product history	
Version	*Modification*
A2	Three reference probes have been replaced compared to version A1.
A1	First release.

Implemented changes in the product description
Version A2-02 – 22 September 2022 (04P) - Product description rewritten and adapted to a new template. - Exon numbering of the *TREX1* has been changed. - Ligation sites of the probes targeting the *TREX1, RNASEH2B, RNASEH2C, RNASEH2A*, and *SAMHD1* genes updated according to new version of the NM_ reference sequence. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. Version A2-01 – 21 February 2019 (01P) - Product description restructured and adapted to a new template. - Transcript variant for *TREX1* has been updated from NM_016381.3 to NM_033629.6.

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