Product Description
SALSA® MLPA® Probemix P229-B5 OPA1

To be used with the MLPA General Protocol.

Version B5
As compared to version B4, one reference probe has been removed. For complete product history see page 8.

Catalogue numbers:
- **P229-025R:** SALSA MLPA Probemix P229 OPA1, 25 reactions.
- **P229-050R:** SALSA MLPA Probemix P229 OPA1, 50 reactions.
- **P229-100R:** SALSA MLPA Probemix P229 OPA1, 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 P229 OPA1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *OPA1* gene, which is associated with optic atrophy type 1. In addition, this probemix contains a probe which detects the wildtype allele of the c.2708delTTAG mutation.

The *OPA1* gene product is a nuclear-encoded mitochondrial protein with similarity to dynamin-related GTPases. It is a component of the mitochondrial network. Mutations in this gene have been associated with optic atrophy type 1, which is a dominantly inherited optic neuropathy resulting in progressive loss of visual acuity, leading in many cases to legal blindness.

The *OPA1* gene (31 exons) spans ~105 kb of genomic DNA and is located on chromosome 3q29, ~195 Mb from the p-telomere (close to the q-telomere).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1248/.

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 *OPA1* exon numbering used in this P229-B5 OPA1 product description is the exon numbering from the LRG_337 sequence. 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 P229-B5 OPA1 contains 40 MLPA probes with amplification products between 130 and 472 nucleotides (nt). This includes 32 probes for the *OPA1* gene. Furthermore, it also contains one probe specific for the wildtype allele of the c.2708delTTAG mutation which will only generate a signal when the wildtype allele is present. 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 optic atrophy type 1. 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. Sample ID number NA03563 from the Coriell Institute has been tested with this P229-B5 probemix at MRC Holland and can be used as a positive control sample to detect a heterozygous duplication of *OPA1*. 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 *OPA1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P229 OPA1.
- 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.

***OPA1* mutation database**

https://databases.lovd.nl/shared/genes/OPA1. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 *OPA1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P229-B5 OPA1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*OPA1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L21056	5q	
142	Reference probe 04120-L03532	17p	
148	**OPA1 probe** 03267-L02704		**Exon 23**
160	**OPA1 probe** 03257-L13646		**Exon 2**
166	**OPA1 probe** 03268-L03713		**Exon 25**
172	**OPA1 probe** 13171-L14919		**Exon 14**
178	**OPA1 probe** 03258-L02695		**Exon 6**
187	**OPA1 probe** 03269-L28147		**Exon 26**
193	Reference probe 01780-L01344	13q	
200	Reference probe 03217-L26776	10q	
208	**OPA1 probe** 13172-L14491		**Exon 13**
214	**OPA1 probe** 03259-L14917		**Exon 7**
221	**OPA1 probe** 13173-L14918		**Exon 3**
229	**OPA1 probe** 06949-L06529		**Exon 30**
238	**OPA1 probe** 03260-L02697		**Exon 8**
247	**OPA1 probe** 13174-L14493		**Exon 4**
256	**OPA1 probe** 03261-L02698		**Exon 10**
265	**OPA1 probe** 03270-L02707		**Exon 28**
273	Reference probe 15957-L28149	6q	
283 ∞ Ж	**OPA1 probe** 14205-SP0118-L14687		**Exon 29**
292	**OPA1 probe** 03262-L02699		**Exon 12**
300 Ø	**OPA1 probe** 13175-L14494		**Intron 16**
310	**OPA1 probe** 13176-L14495		**Exon 9**
319	**OPA1 probe** 20377-L28150		**Exon 16**
328	**OPA1 probe** 06944-L06524		**Exon 1**
337	**OPA1 probe** 03264-L02701		**Exon 19**
346	Reference probe 18927-L24522	1p	
363 Ø	**OPA1 probe** 13170-L14701		**Intron 29**
373	**OPA1 probe** 03265-L02702		**Exon 20**
382	**OPA1 probe** 13177-L14702		**Exon 18**
392	**OPA1 probe** 13178-L14497		**Exon 21**
400	**OPA1 probe** 03266-L02703		**Exon 22**
409	**OPA1 probe** 03272-L02709		**Exon 31**
420	**OPA1 probe** 20378-L28151		**Exon 5**
427	**OPA1 probe** 13180-L14499		**Exon 27**
434	**OPA1 probe** 06948-L14438		**Exon 24**
445	**OPA1 probe** 06946-L14439		**Exon 11**
454 Ø	**OPA1 probe** 12629-L14440		**Intron 1**
463	Reference probe 20114-L27638	8q	
472	Reference probe 18501-L25035	19q	

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

∞ Wild type sequence detected. A lowered probe signal can be due to a c.2708delTTAG mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

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.

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.

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. *OPA1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*OPA1* exon[a]	Ligation site NM_130837.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*171-173 (Exon 1)*		
328	06944-L06524	Exon 1	88 nt after exon 1	AAATGTGCAGGT-GACTCTCAGGCC	20.7 kb
454 Ø	12629-L14440	Intron 1	550 nt before exon 2	GATTGATTAACT-TCCTTAATTTCC	0.8 kb
160	03257-L13646	Exon 2	462-463	CTACGAGACTCT-TAAAACTTCGCT	0.7 kb
221	13173-L14918	Exon 3	556-557	GATACCGGACCT-TAGTGAATATAA	1.5 kb
247	13174-L14493	Exon 4	688-689	TGACAAGATTGT-TGAAAGCCTTAG	0.6 kb
420	20378-L28151	Exon 5	8 nt after exon 5	TTAGGTGTGTAA-ACAGACATTTTT	1.0 kb
178	03258-L02695	Exon 6	789-790	AAGGTTCTCCGG-AAGAAACGGCGT	7.2 kb
214	03259-L14917	Exon 7	874-875	GCTCATTCTCTT-ACAACAACAAAT	5.5 kb
238	03260-L02697	Exon 8	1002-1001 *reverse*	CTGAGTGTGCAG-AAGTTCTTCCTG	3.9 kb
310	13176-L14495	Exon 9	17 nt after exon 9	CTAAGTTTGTCT-TGTTTATTCTCA	1.7 kb
256	03261-L02698	Exon 10	1176-1175 *reverse*	CGTATTATAACT-GGCATCATAATC	0.8 kb
445	06946-L14439	Exon 11	1304-1305	GAGATGATGACA-CGTTCTCCAGTT	4.7 kb
292	03262-L02699	Exon 12	1329-1330	AGGTGACTCTGA-GTGAAGGTCCTC	0.3 kb
208	13172-L14491	Exon 13	3 nt after exon 13	AGCCCTGAGGTA-AGGGTTGCAATT	0.3 kb
172	13171-L14919	Exon 14	1516-1515 *reverse*	CAACAAGCACCA-TCCTCTGTAGTC	0.6 kb
	No probe	Exon 15			
319	20377-L28150	Exon 16	1692-1693	CAGACTTGGTCA-GTCAAATGGACC	0.9 kb
300 Ø	13175-L14494	Intron 16	609 nt before exon 17 *reverse*	TTTCACATATGT-TAGAGTTTCTAC	1.1 kb
	No probe	Exon 17			
382	13177-L14702	Exon 18	203 nt after exon 18 *reverse*	TGCCAGTATGTA-TTGTACCATGTG	1.2 kb
337	03264-L02701	Exon 19	1999-2000	CTTTTGGAAAAT-GGTACGAGAGTC	1.0 kb
373	03265-L02702	Exon 20	2067-2068	TTGAAACTGAAT-GGAAGAATAACT	0.7 kb
392	13178-L14497	Exon 21	37 nt before exon 21	AATTTACTGTCT-TATGGAAATCTT	6.2 kb
400	03266-L02703	Exon 22	2236-2237	TCATGTGATTGA-AAACATCTACCT	2.2 kb
148	03267-L02704	Exon 23	2377-2378	ACAAGAAGAATT-TTCCCGCTTTAT	1.8 kb
434	06948-L14438	Exon 24	2560-2561	ACAGCAATGGGA-TGCAGCTATTTA	0.6 kb
166	03268-L03713	Exon 25	2639-2640	AACATGGTGGGT-CCAGACTGGAAA	3.4 kb
187	03269-L28147	Exon 26	2761-2762	CCCAGCTTATCT-TGCAAGTGATGA	1.7 kb
427	13180-L14499	Exon 27	290 nt before exon 27 *reverse*	AGTAGACAGGGT-AGGTATTACCTT	1.7 kb
265	03270-L02707	Exon 28	2975-2976	GTGGTCTTGTTT-TGGCGTATACAG	0.8 kb
283 ∞ Ж	14205-SP0118-L14687	Exon 29	3043-3044; 3077-3078	TCTTTAAACAGT-GAAGATTTTGCT	13.4 kb
363 Ø	13170-L14701	Intron 29	11483 nt before exon 30 *reverse*	AAGAGGTAGGCA-TCATTTATCACC	11.7 kb
229	06949-L06529	Exon 30	104 nt after exon 30	CTTGTCACTTAT-GCTGTAATTTCA	2.5 kb
409	03272-L02709	Exon 31	3380-3381	CTTTAACCATCA-GCTGCCTCTCGA	
		stop codon	*3216-3218 (Exon 30)*		

[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.

∞ Wild type sequence detected. A lowered probe signal can be due to a c.2708delTTAG mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

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.

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.

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.

Related SALSA MLPA probemixes

- P219 PAX6: Contains probes for the *PAX6*, *SOX2* and *WT1* genes involved in hereditary ocular malformations.
- P221 LCA1 mix-1: Contains probes for the *AIPL1*, *CRB1*, *CRX* and *RPE65* genes involved in Leber congenital amaurosis.
- P222 LCA2 mix-2: Contains probes for the *GUCY2D*, *RDH12*, *RPGRIP1* and *CEP290* genes involved in Leber congenital amaurosis.
- P367 BEST1-PRPH2: Contains probes for the *BEST1* and *PRPH2* genes involved in vitelliform macular dystrophy.

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 P229 OPA1

- Almind GJ et al. (2011). Genomic deletions in OPA1 in Danish patients with autosomal dominant optic atrophy. *BMC Med Genet*. 12:49.
- Bolognini R et al. (2017). Characterization of two novel intronic OPA1 mutations resulting in aberrant pre-mRNA splicing. *BMC Med Genet*. 18:22.
- Galvez-Ruiz A et al. (2013). First cases of dominant optic atrophy in Saudi Arabia: report of two novel OPA1 mutations. *J Neuroophthalmol*. 33:349-353.
- Charif M et al. (2015). A novel mutation of AFG3L2 might cause dominant optic atrophy in patients with mild intellectual disability. *Front Genet*. 6:311.
- Chen J (2014). Mutation screening of mitochondrial DNA as well as OPA1 and OPA3 in a Chinese cohort with suspected hereditary optic atrophy. *Invest Ophthalmol Vis Sci*. 55:6987-95.
- Fuhrmann N et al. (2010). Solving a 50 year mystery of a missing OPA1 mutation: more insights from the first family diagnosed with autosomal dominant optic atrophy. *Mol Neurodegener*. 5:25.
- Gaier ED et al. (2017). Diagnostic genetic testing for patients with bilateral optic neuropathy and comparison of clinical features according to OPA1 mutation status. *Mol Vis*. 23:548-60.
- Hayashi T et al. (2017). Heterozygous deletion of the OPA1 gene in patients with dominant optic atrophy. *Jpn J Ophthalmol*. 61:395-401.

P229 product history	
Version	*Modification*
B5	One reference probe has been removed.
B4	Three reference probes have been replaced.
B3	Three reference probes have been replaced.
B2	Two reference probes and the 88 and 96 nt control fragments have been replaced (QDX2).
B1	Probes for *VMD2* and *RDS* have been removed, 18 new OPA1 probes have been added, 2 *OPA1* probes have been exchanged.
A2	Four extra control fragments added at 88-96-100-105 nt.
A1	First release.

Implemented changes in the product description
Version B5-01 – 18 January 2022 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the OPA1 gene updated according to new version of the NM_ reference sequence. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Removed probemix P149 from 'Related probemixes' since this product has been discontinued. Version B4-01 – 03 July 2018 (01P) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200