

Product Description SALSA® MLPA® Probemix P081-D1 NF1 mix 1 & P082-C2 NF1 mix 2

To be used with the MLPA General Protocol.

P081 version D1. As compared to version C1, exon 21 and exon 23 probes have been replaced, a probe for exon 21 has been added, a reference probe has been replaced and several probes have a small change in length. For complete product history see page 10.

P082 version C2. As compared to version C1, two reference probes have been replaced and one probe has a small change in length. For complete product history see page 10.

Catalogue numbers:

- **P081-025R:** SALSA MLPA probemix P081 NF1 mix 1, 25 reactions.
- **P081-050R:** SALSA MLPA probemix P081 NF1 mix 1, 50 reactions.
- **P081-100R:** SALSA MLPA probemix P081 NF1 mix 1, 100 reactions.

- **P082-025R:** SALSA MLPA probemix P082 NF1 mix 2, 25 reactions.
- **P082-050R:** SALSA MLPA probemix P082 NF1 mix 2, 50 reactions.
- **P082-100R:** SALSA MLPA probemix P082 NF1 mix 2, 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.

Copy number variation (CNV) analysis of all exons of the *NF1* gene requires the use of both SALSA MLPA probemixes, P081 NF1 mix 1 and P082 NF1 mix 2.

Intended use: The SALSA MLPA probemixes P081 NF1 mix 1 and P082 NF1 mix 2 are an in vitro diagnostic (IVD)¹ or a research use only (RUO) assay for the detection of deletions or duplications in the human *NF1* gene in order to confirm a clinical diagnosis of Neurofibromatosis type 1. This assay is for use with human DNA derived from peripheral blood and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials.

Deletions or duplications detected with the P081 NF1 mix 1 and P082 NF1 mix 2 probemixes should be verified by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in the *NF1* gene are point mutations, which will not be detected by MLPA. It is therefore recommended to use these SALSA MLPA probemixes in combination with sequence analysis of the *NF1* gene. These probemixes are not intended to be used as standalone assays for clinical decisions. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

¹Please note that these probemixes are for In Vitro Diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Neurofibromatosis is an autosomal dominant disorder characterised particularly by café-au-lait spots and fibromatous tumours of the skin. Neurofibromatosis type 1 is caused by loss-of-function mutations in the *NF1* gene on 17q11.2. Neurofibromatosis type 2 is caused by defects in the *NF2* gene on chromosome 22q12.2, for which the P044 NF2 MLPA probemix (RUO) can be used.

Estimated birth incidence of Neurofibromatosis type 1 is 1 in 3000, with about half of the NF1 cases caused by *de novo* sporadic mutations. *De novo* sporadic mutations may also be the result of germline mosaicism in apparently unaffected parents.

Deletions of part of the *NF1* gene as well as deletions and duplications of the complete *NF1* gene have been described. Relatively common (5-10% of NF1 cases) is a deletion of a 1.4 Mb chromosomal region harbouring multiple genes, including the *NF1* gene. The phenotype of this 17q11.2 microdeletion is usually much more severe than most other NF1 cases and may include developmental delay. Next to the 1.4 Mb deletion described above, a 1.2 Mb microdeletion and nonrecurrent atypical microdeletions of different sizes have been reported. The P122 NF1 area MLPA probemix (RUO) can be used to determine the extent of the deletion as it contains many probes for other genes in the frequently deleted 1.4 Mb region.

More information is available on <https://www.ncbi.nlm.nih.gov/books/NBK1109/>.

Gene structure: The *NF1* gene spans ~300 kb on chromosome 17q11.2. The *NF1* LRG_214 sequence is available at <http://www.lrg-sequence.org/> and is identical to the NCBI NG_009018.1 sequence.

Transcript variants: <https://www.ncbi.nlm.nih.gov/gene/4763> describes several transcript variants of the *NF1* gene. The NM_000267.3 sequence represents transcript variant 2. This sequence is a reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 1 (384-386) and the stop codon is located in exon 58 (8838-8840). The NM_001042492.2 sequence represents transcript variant 1. This transcript variant contains an additional in-frame coding exon (31).

Exon numbering: The exon numbering used in this P081-D1/P082-C2 NF1 product description is the exon numbering from the NCBI NG_009018.1 which is identical to the LRG_214 sequence. The exon numbering and NM_ sequence used have been retrieved on 03/2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date. Additional information on exon numbering corresponding to the different NM sequences is available in the Table included in Appendix I of this product description.

P081-D1 and P082-C2 probemix content: The P081 and P082 probemixes together contain one probe for each exon, three probes for exon 1, one probe for intron 1, and two probes for the exons 15, 21, 23, 51 and 58 of the *NF1* gene. Additionally, these probemixes contain one upstream and one downstream probe and two probes for the *OMG* gene, located within intron 36 of the *NF1* gene.

The P081-D1 probemix contains 46 MLPA probes with amplification products between 130 and 463 nt in length, including 11 reference probes. The P082-C2 probemix contains 44 MLPA probes with amplification products between 130 and 483 nt in length, nine of which are reference probes. The identity of the genes detected by the reference probes is available online (www.mlpa.com).

Both these probemixes contain 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 and 96 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 from peripheral blood, 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 Neurofibromatosis type 1. 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.

Performance characteristics: The expected number of *NF1* deletions/duplications which can be detected with these MLPA probemixes is approximately 10% of all *NF1* mutations in most patient populations. Analytical performance for the detection of deletions/duplications in the *NF1* gene is very high and can be considered >99% (based on a 2006-2018 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software must 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 expected results for the *NF1* specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication).

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 or in or near the *NF1* gene. 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 in some cases 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.

P081/P082 specific notes:

- Due to the presence of pseudogenes, probe design in the *NF1* gene is difficult. The P081/P082 probemixes were designed to be specific for the *NF1* gene. This specificity was confirmed by testing of a positive sample with an *NF1* deletion. In rare cases, if changes in the pseudogenes occur, apparent duplications might be detected.
- Mosaicism has been reported in individuals with NF1. Mosaic *NF1* deletions obtained with the P081/P082 NF1 probemixes must be confirmed by analysis of a second, independently collected DNA sample or a different technique, in order to exclude a false positive mosaic result.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *NF1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemixes P081/P082 NF1.
- 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 one or 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.

***NF1* mutation database:** <http://www.LOVD.nl/NF1>. We strongly encourage users to deposit positive results in the LOVD 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 SNPs and unusual results (e.g., a duplication of *NF1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P081-D1 NF1 mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^(a)	
		Reference	NF1
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
136 « ↯	NF1 probe 18363-L23328		Downstream
142 ¥	NF1 probe 02491-L29974		Exon 1
148	NF1 probe 18364-L23329		Exon 28
154	NF1 probe 05220-L03309		Exon 57
160	NF1 probe 02493-L01924		Exon 2
166	NF1 probe 02513-L01944		Exon 32
172	Reference probe 09940-L29795	8q13	
178	NF1 probe 02865-L02617		Exon 4
184	NF1 probe 18367-L23332		Exon 35
190	Reference probe 09836-L10246	11q24	
196 ↯	NF1 probe 18368-L23333		Upstream
202	NF1 probe 02497-L03706		Exon 6
208 «	NF1 probe 19361-L26126		Exon 58
214	NF1 probe 02517-L26127		Exon 37
220	NF1 probe 18032-L22398		Exon 7
226	NF1 probe 19363-L25737		Exon 51
232	NF1 probe 13221-L26128		Exon 11
238	NF1 probe 02519-L01950		Exon 39
244 *	NF1 probe 21185-L29794		Exon 21
250 ¥	NF1 probe 03849-L18072		Exon 26
256 ¥ Ж	NF1 probe 18033-SP0601-L29798		Exon 24
264	Reference probe 09265-L10877	10q21	
272	NF1 probe 02521-L22646		Exon 41
279	Reference probe 12437-L13438	14q24	
289	NF1 probe 04071-L01954		Exon 47
298	NF1 probe 02503-L22647		Exon 13
304	Reference probe 16436-L18889	18q21	
312	NF1 probe 04076-L22649		Exon 15
319	NF1 probe 02525-L22650		Exon 49
328	Reference probe 05388-L04785	12p11	
337 *	NF1 probe 21000-L29222		Exon 23
346	NF1 probe 02526-L01957		Exon 50
353	NF1 probe 02507-L22658		Exon 17
364 *	Reference probe 05953-L05397	2p22	
373	NF1 probe 02528-L01959		Exon 52
382 *	NF1 probe 21186-L29799		Exon 21
391 «	NF1 probe 02530-L01961		Exon 58
400	NF1 probe 04072-L03709		Exon 29
409	Reference probe 08725-L08736	9q21	
418 Ж	NF1 probe 18408-SP0653-L23405		Exon 23
427	NF1 probe 12024-L26426		Exon 18
436	NF1 probe 03853-L29796		Exon 42
445	Reference probe 05026-L29797	2q32	
454 Ø	OMG probe 04075-L03310		Intron 36 of NF1 (OMG gene)
463	Reference probe 09908-L10321	16p13	

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

* New in version D1 (from lot D1-0617 onwards).

¥ Changed in version D1 (from lot D1-0617 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.

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. Be aware that when this occurs in reference samples, it can look like an increased signal in the test samples.

→ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

∅ Probe detects the *OMG* gene, located within intron 36 of *NF1* gene. We have no information on the clinical significance of copy number changes of only the *OMG* gene.

Table 1b. SALSA MLPA Probemix P082-C2 NF1 mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^(a)	
		Reference	<i>NF1</i>
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
138 Ж	NF1 probe 18382-L19008		Exon 1
147	NF1 probe 02512-L01943		Exon 30
154	NF1 probe 12018-L12866		Exon 53
160	NF1 probe 02494-L01925		Exon 3
166	NF1 probe 02514-L01945		Exon 34
172	NF1 probe 18173-L22738		Exon 5
178	Reference probe 11571-L12318	16q21	
184	Reference probe 17862-L22121	19q13	
190	NF1 probe 12019-L12867		Intron 1
197	NF1 probe 18374-L26502		Exon 36
205	NF1 probe 02498-L22716		Exon 8
211	NF1 probe 02518-L01949		Exon 38
220	Reference probe 12427-L13428	22q12	
227	NF1 probe 19362-L26201		Exon 1
233	NF1 probe 02500-L26202		Exon 10
241	NF1 probe 02520-L26200		Exon 40
249	NF1 probe 12021-L26199		Exon 44
257	NF1 probe 03778-L26198		Exon 12
265	NF1 probe 02522-L01953		Exon 46
271	Reference probe 15957-L26197	6q15	
281 Ж	NF1 probe 19364-SP0809-L25738		Exon 15
292	NF1 probe 02504-L26817		Exon 14
300	NF1 probe 02524-L22720		Exon 48
307	NF1 probe 18034-L22721		Exon 54
317 Ж	NF1 probe 18369-SP0646-L23334		Exon 16
328	NF1 probe 13217-L22725		Exon 51
337	NF1 probe 18370-L23335		Exon 20
345	NF1 probe 02529-L01960		Exon 55
353	Reference probe 06708-L26176	10p11	
362 Ж	NF1 probe 18174-SP0619-L22739		Exon 25
372	Reference probe 08893-L23475	14q24	
382	NF1 probe 18035-L22401		Exon 56
391	NF1 probe 18365-L23330		Exon 33
400 *	Reference probe 07808-L23525	3p22	
409	NF1 probe 18170-L26175		Exon 27
419	NF1 probe 03854-L23156		Exon 43
427 ∆	NF1 probe 12025-L23157		Exon 19
436	NF1 probe 18036-L22765		Exon 22
444 ∅	OMG probe 04069-L03311		Intron 36 of <i>NF1</i> (<i>OMG</i> gene)
454	NF1 probe 03856-L03307		Exon 45
463 Ж	NF1 probe 18037-SP0602-L22403		Exon 9
472	NF1 probe 18038-L26174		Exon 31
483 *	Reference probe 06676-L06254	11p15	

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

* New in version C2 (from lot C2-0317 onwards).

‡ Changed in version C2 (from lot C2-0317 onwards). Small change in length, no change in sequence detected.

∅ Probe detects the *OMG* gene, located within intron 36 of *NF1* gene. We have no information on the clinical significance of copy number changes of only the *OMG* gene.

⋈ 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. Be aware that when this occurs in reference samples, it can look like an increased signal in the test samples.

Δ More variable. This probe is sensitive to experimental conditions. Aberrant results should be treated with caution.

Table 2. NF1 probes arranged according to chromosomal location

Length (nt)		SALSA MLPA probe	NF1 exon ^(a)	Ligation site ^(b) NM_000267.3	Partial sequence ^(c) (24 nt adjacent to ligation site)	Distance to next probe
P081	P082					
			<i>start codon</i>	<i>384-386 (ex 1)</i>		
196 ↵		18368-L23333	Upstream	8.0 kb before exon 1	CAAAGCAAGTTC-AGCATCAGAGGA	7.7 kb
142		02491-L29974	Exon 1	335 nt before exon 1	GCAGAGATCCGC-GCGCTGGGAGAA	0.4 kb
	227	19362-L26201	Exon 1	53-54	AAGGATCCCACT-TCCGGTGGGGTG	0.3 kb
	138	18382-L19008	Exon 1	415-414 reverse	TGACCACGGCCT-GGACCCATTCCA	0.6 kb
	190	12019-L12867	Intron 1	597 nt after exon 1	TCGTCTCATCCT-GCCCCGAGAGCT	60.1 kb
160		02493-L01924	Exon 2	475-476	GCAGAACACACA-TACCAAAGTCAG	3.0 kb
	160	02494-L01925	Exon 3	631-632	ATATCTCTCTCA-GTTGATTATATT	4.2 kb
178		02865-L02617	Exon 4	735-736	TGCCAGAAATCT-GCCATTTTCTTC	6.7 kb
	172	18173-L22738	Exon 5	958-959	AAAATTAACACG-ACTCCTGAAGGG	11.5 kb
202		02497-L03706	Exon 6	1000-1001	AGCCCTAAAGAA-GGTTGCGCAGTT	0.3 kb
220		18032-L22398	Exon 7	1046-1047	TAGGCATTTTGG-AACTGGGTAGAA	0.9 kb
	205	02498-L22716	Exon 8	1167-1168	AAAGCACCAAAC-GTAAAGCAGCAG	17.9 kb
	463 ⋈ #	18037-SP0602-L22403	Exon 9	1341-1342; 1372-1373	TGACAGAAAGTG-31 nt spanning oligo-AAGTACTTACAT	0.6 kb
	233 #	02500-L26202	Exon 10	1508-1509	GATGTGGATCTA-ATGATTGACTGC	0.7 kb
232 #		13221-L26128	Exon 11	291 nt after exon 11	TGAGAAAAATGT-CACTGAAAATAC	4.5 kb
	257	03778-L26198	Exon 12	1722-1723	TTGGTGAAACAC-TTCATAAAGCAG	8.2 kb
298 #		02503-L22647	Exon 13	1849-1850	GACAAGAAAGCTA-TAAGTATCTTCT	4.6 kb
	292	02504-L26817	Exon 14	1985-1986	CAACTGGTCCCT-CAGTCACACATG	2.5 kb
312		04076-L22649	Exon 15	235 nt before exon 15 reverse	TAAGTGGCATGT-ACATATAAAGCT	0.3 kb
	281 ⋈ #	19364-SP0809-L25738	Exon 15	2053-2054; 2083-2084	TCAGTTAGATAG-30 nt spanning oligo-AGAAACATTTTG	1.5 kb
	317 ⋈ #	18369-SP0646-L23334	Exon 16	35 nt before exon 16; 2107-2108	TTAGGTTATTGA-38 nt spanning oligo-ACAAATGCTTTT	1.8 kb
353		02507-L22658	Exon 17	2329-2330	GGATCATGAAGA-ATTACTACGTAC	1.4 kb
427 #		12024-L26426	Exon 18	2587-2588	CTTGCCCAACTA-TAACACATTCAT	0.7 kb
	427 # Δ	12025-L23157	Exon 19	2 nt after exon 19	AACACTGAGGTA-TGCCCTTAGCAA	0.2 kb
	337	18370-L23335	Exon 20	34 nt before exon 20	AGCTCTAGACTA-AGTTGCTTTCAA	1.8 kb
244 #		21185-L29794	Exon 21	3064-3063 reverse	GCCGATCCATAA-ATTTGCTGACAG	0.1 kb
382 #		21186-L29799	Exon 21	3170-3171	AGTCCTGCTCTG-TATCCAATGCTA	0.4 kb
	436 #	18036-L22765	Exon 22	3269-3270	CAATTTGTAGAA-CAAACCATAGCT	0.5 kb
418 ⋈ #		18408-SP0653-L23405	Exon 23	3434-3435; 3468-3469	AAACTGTGTCAA-34 nt spanning oligo-TCTCATTTTGCC	0.2 kb
337		21000-L29222	Exon 23	179 nt after exon 23	CCTGTGACAATG-CTCCCTTTTCT	0.2 kb
256 ⋈ #		18033-SP0601-L29798	Exon 24	37 nt before exon 24; 3498-3499	GGCTTCAAAAAC-39 nt spanning oligo-ATAAGATGGTAG	1.4 kb
	362 ⋈	18174-SP0619-L22739	Exon 25	3681-3682; 34 nt after exon 25	TGGAAGCCAAAT-51 nt spanning oligo-GCAAATAAAGCC	0.6 kb
250 #		03849-L18072	Exon 26	3816-3817	TGAGGCACTGTA-CGGTCCTTGCAA	0.3 kb
	409	18170-L26175	Exon 27	4002-4003	ATCGGTTTGAGA-GATTGGTGAAC	2.6 kb
148		18364-L23329	Exon 28	4196-4197	GCAGACTCCATG-CAGACTCTCTTC	0.3 kb

Length (nt)		SALSA MLPA probe	NF1 exon ^(a)	Ligation site ^(b) NM_000267.3	Partial sequence ^(c) (24 nt adjacent to ligation site)	Distance to next probe
P081	P082					
400 #		04072-L03709	Exon 29	4323-4324	CATCCTCTGATT-GGCAACATGTTA	13.0 kb
	147	02512-L01943	Exon 30	4390-4391	TGAGAAAACCA-GCGGAACCTCCT	3.9 kb
	472	18038-L26174	Exon 31	NM_001042492.2: 4497-4498	TTCTGTAGCAA-CTTGCCACTCCC	5.5 kb
166 #		02513-L01944	Exon 32	4534-4535	CATCGGTGCACT-AGGAAGTGCCAT	0.7 kb
	391 #	18365-L23330	Exon 33	4732-4733	TGTGAAAAGCAA-CTTTGATGCAGC	1.3 kb
	166	02514-L01945	Exon 34	4816-4817	TCTTTCCTTCAT-AAGTGACGGCAA	1.3 kb
184 #		18367-L23332	Exon 35	4958-4959	CTTGCATACCTG-GGTCCTCCAGAG	3.4 kb
	197 #	18374-L26502	Exon 36	12 nt before exon 36	ATTACTCTGTTA-TTTTTCTTTTAG	30.3 kb
	444 ∅	04069-L03311	OMG gene; Exon 2	NM_002544.5: 950-951, within NF1 intron 36	GCAGACAGTGGA-CACCATTAATC	0.6 kb
454 ∅		04075-L03310	OMG gene; Exon 2	NM_002544.5: 384-383 reverse, within NF1 intron 36	CACAGAGACCGA-GGTAAGTGAGCA	30.1 kb
214		02517-L26127	Exon 37	5427-5428	GCCTCAAAGGTA-GCAAAGGCTTG	1.5 kb
	211	02518-L01949	Exon 38	5720-5721	AACCAGTTCACC-TTAACCATTGCA	2.7 kb
238		02519-L01950	Exon 39	6008-6009	CTAGAGACATCA-GGTTTATGTATC	4.5 kb
	241	02520-L26200	Exon 40	6178-6179	GACTCCATGGCT-GTCAAATCTAGT	1.5 kb
272		02521-L22646	Exon 41	6390-6391	CAGGTGGCTTGG-GATCAATAAAAG	0.4 kb
436		03853-L29796	Exon 42	6592-6593	GCTGTCCTCAA-CAATTCCTTGA	0.7 kb
	419	03854-L23156	Exon 43	6800-6801	TCATTACCCAAA-TTTTACTTGCTG	0.4 kb
	249	12021-L26199	Exon 44	6989-6990	ATTCCAACGTGC-AAGTGGCTGGAC	0.2 kb
	454	03856-L03307	Exon 45	7075-7076	TCTTGTGTCTT-TGGGTGTATTAG	0.6 kb
	265	02522-L01953	Exon 46	7162-7163	TTGCTTAAAGG-ACCTGACACTTA	1.8 kb
289		04071-L01954	Exon 47	7269-7270	AAGCCCTTTTT-GGGTAGCTGTGG	2.5 kb
	300	02524-L22720	Exon 48	7425-7426	ATCCTCTGGAGT-GGCACTGCAAGC	6.1 kb
319		02525-L22650	Exon 49	7535-7536	TCACCTGCTATT-GTTGCAAGAACA	1.0 kb
346		02526-L01957	Exon 50	7671-7672	AAGAAGTTCGAA-GTCGCTGCAGCC	2.1 kb
	328	13217-L22725	Exon 51	7799-7800	GAGACTCAGCCA-TGGTCTCTCCC	0.1 kb
226		19363-L25737	Exon 51	7860-7861	CTGTCGGCCAGA-CCAGTCCCCGAG	4.2 kb
373		02528-L01959	Exon 52	7997-7998	AGGCAAGAAATG-GAATCAGGGATC	0.5 kb
	154	12018-L12866	Exon 53	8107-8108	TTTACGTAAAGT-TTCAGTGTCTGA	0.3 kb
	307	18034-L22721	Exon 54	8229-8230	AGTTTGATCAAC-GAATCTTTTATG	1.3 kb
	345	02529-L01960	Exon 55	8386-8387	GCAGAGTGTGGT-GTACCATGAAGA	0.4 kb
	382	18035-L22401	Exon 56	2 nt before exon 56	TTGATTTGTTGC-AGGTTTTGGTTT	1.6 kb
154		05220-L03309	Exon 57	8563-8564	TGGAATTGATGA-AGAAACCAAGTGA	13.5 kb
391 «		02530-L01961	Exon 58	8748-8749	GCCACTGTAACA-GTGGACCAACTC	2.0 kb
208 «		19361-L26126	Exon 58	10796-10797	AGTGCCAAGGAT-GCCAAGCTGCCA	6.4 kb
136 « ↵		18363-L23328	Downstream	4.8 kb after exon 58	GGGAAGGAGCTC-AGGCTGTAATGT	
				<i>stop codon</i>	<i>8838-8840 (ex 58)</i>	

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

(b) Ligation sites of the P081/P082 NF1 MLPA probes are indicated according to RefSeq sequence NM_000267.3 containing 57 exons, with the exception of the probe for exon 31. Ligation site of the last mentioned probe is indicated according to RefSeq sequence NM_001042492.2, containing 58 exons. Additional information on exon numbering and ligation sites corresponding to the different NM sequences is available in the Table included in Appendix I of this product description.

(c) 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.

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. Be aware that when this occurs in reference samples, it can look like an increased signal in the test samples.

↪ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

∅ Probe detects the *OMG* gene, located within intron 36 of *NF1* gene. We have no information on the clinical significance of copy number changes of only the *OMG* gene.

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.

Δ More variable. This probe is sensitive to experimental conditions. Aberrant results should be treated with caution.

Related SALSA MLPA probemixes

P044 NF2:	Contains probes for the <i>NF2</i> gene, involved in Neurofibromatosis type 2.
P122 NF1 area:	Contains probes for the 17q11.2 region, involved in Neurofibromatosis type 1.
P295 SPRED1:	Contains probes for the <i>SPRED1</i> gene at 15q14, involved in Neurofibromatosis type 1-like syndrome.

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P081 Product history	
<i>Version</i>	<i>Modification</i>
D1	Exon 21 and exon 23 probes have been replaced, a probe for exon 21 has been added, a reference probe has been replaced, and several probes have a small change in length.
C1	Eleven target probes have been added or replaced and ten references have been added or replaced.
B2	Control fragments have been adjusted.
B1	Three NF1 probes and two reference probes have been replaced and two new control fragments at 100-105 nt have been included.
A1	First release.

P082 Product history	
<i>Version</i>	<i>Modification</i>
C2	Two reference probes have been replaced and one probe has a small change in length.
C1	Thirteen target probes have been added or replaced and eight references have been added or replaced.
B2	Control fragments have been adjusted.
B1	Six NF1 probes and five reference probes have been replaced and two new control fragments at 100-105 nt have been included.
A2	One reference probe has been replaced.
A1	First release.

Implemented changes in the product description

Version D1/C2-04 – 22 April 2020 (04)

- Product is now registered for IVD use in Colombia.

Version D1/C2-03 – 10 April 2019 (04)

- The sections precautions and warnings, exon numbering, interpretation of results, and confirmation of results were updated.
- Warning added to Table 1b and Table 2 for more variable probe, 427 nt probe 12025-L23157.
- Ligation sites of probes targeting the OMG gene were updated according to new version of the NM_reference sequence.
- Five references using the P081/P082 probemixes have been added to the section selected publications.
- Additional information on exon numbering corresponding to the different NM sequences is available in the Table included in Appendix I of this product description.
- Product is now registered for IVD use in Morocco and Israel.
- Minor textual and layout changes.

Version D1/C2-02 – 03 July 2018 (04)

- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Information about Positive control DNA samples was adjusted.
- Product description adapted to a new template.
- Minor textual and layout changes.

Version P081-D1/P082-C2-01 – 06 July 2017 (03)

- 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).
- Remarks about variability and SNPs removed from information at Table 1.

Version 30 – 06 June 2017 (55)

- Product description adapted to a new product lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Information about NF1 gene and common microdeletion adjusted on page 1.
- Minor textual and layout changes.

Version 29 – 31 July 2015 (54)



- Warnings added in Table 1 and Table 2. Low signals for probes that consist of three parts can be due to depurination of the sample DNA.

Version 28 (53)

- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- New references added on page 1.

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

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	EUROPE* CE MOROCCO ISRAEL COLOMBIA
	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA).
The product is for RUO in all other European countries.

Appendix I: Additional information on *NF1* exon numbering

The table below, describes the *NF1* exon numbering and ligation sites, based on NM_000267.3 and NM_001042492.2.

Length (nt)		SALSA MLPA probe	<i>NF1</i> exon based on LRG 214	<i>NF1</i> exon numbering and ligation site based on NM_000267.3	<i>NF1</i> exon numbering and ligation site based on NM_001042492.2
				<i>start codon</i> 384-386 (ex 1)	<i>start codon</i> 384-386 (ex 1)
196	↵	18368-L23333	Upstream	Upstream 8.0 kb before exon 1	Upstream 8.0 kb before exon 1
142		02491-L29974	Exon 1	Exon 1 335 nt before exon 1	Exon 1 335 nt before exon 1
	227	19362-L26201	Exon 1	Exon 1 53-54	Exon 1 53-54
	138	18382-L19008	Exon 1	Exon 1 415-414 reverse	Exon 1 415-414 reverse
	190	12019-L12867	Intron 1	Intron 1 597 nt after exon 1	Intron 1 597 nt after exon 1
160		02493-L01924	Exon 2	Exon 2 475-476	Exon 2 475-476
	160	02494-L01925	Exon 3	Exon 3 631-632	Exon 3 631-632
178		02865-L02617	Exon 4	Exon 4 735-736	Exon 4 735-736
	172	18173-L22738	Exon 5	Exon 5 958-959	Exon 5 958-959
202		02497-L03706	Exon 6	Exon 6 1000-1001	Exon 6 1000-1001
220		18032-L22398	Exon 7	Exon 7 1046-1047	Exon 7 1046-1047
	205	02498-L22716	Exon 8	Exon 8 1167-1168	Exon 8 1167-1168
	463 Ж #	18037-SP0602-L22403	Exon 9	Exon 9 1341-1342; 1372-1373	Exon 9 1341-1342; 1372-1373
	233 #	02500-L26202	Exon 10	Exon 10 1508-1509	Exon 10 1508-1509
232 #		13221-L26128	Exon 11	Exon 11 291 nt after exon 11	Exon 11 291 nt after exon 11
	257	03778-L26198	Exon 12	Exon 12 1722-1723	Exon 12 1722-1723
298 #		02503-L22647	Exon 13	Exon 13 1849-1850	Exon 13 1849-1850
	292	02504-L26817	Exon 14	Exon 14 1985-1986	Exon 14 1985-1986
312		04076-L22649	Exon 15	Exon 15 235 nt before exon 15 reverse	Exon 15 235 nt before exon 15 reverse
	281 Ж #	19364-SP0809-L25738	Exon 15	Exon 15 2053-2054; 2083-2084	Exon 15 2053-2054; 2083-2084
	317 Ж #	18369-SP0646-L23334	Exon 16	Exon 16 35 nt before exon 16; 2107-2108	Exon 16 35 nt before exon 16; 2107-2108
353		02507-L22658	Exon 17	Exon 17 2329-2330	Exon 17 2329-2330
427 #		12024-L26426	Exon 18	Exon 18 2587-2588	Exon 18 2587-2588
	427 # Δ	12025-L23157	Exon 19	Exon 19 2 nt after exon 19	Exon 19 2 nt after exon 19
	337	18370-L23335	Exon 20	Exon 20 34 nt before exon 20	Exon 20 34 nt before exon 20
244 #		21185-L29794	Exon 21	Exon 21 3064-3063 reverse	Exon 21 3064-3063 reverse
382 #		21186-L29799	Exon 21	Exon 21 3170-3171	Exon 21 3170-3171
	436 #	18036-L22765	Exon 22	Exon 22 3269-3270	Exon 22 3269-3270
418 Ж #		18408-SP0653-L23405	Exon 23	Exon 23 3434-3435; 3468-3469	Exon 23 3434-3435; 3468-3469
337		21000-L29222	Exon 23	Exon 23 179 nt after exon 23	Exon 23 179 nt after exon 23
256 Ж #		18033-SP0601-L29798	Exon 24	Exon 24 37 nt before exon 24; 3498-3499	Exon 24 37 nt before exon 24; 3498-3499
	362 Ж	18174-SP0619-L22739	Exon 25	Exon 25 3681-3682; 34 nt after exon 25	Exon 25 3681-3682; 34 nt after exon 25
250 #		03849-L18072	Exon 26	Exon 26 3816-3817	Exon 26 3816-3817
	409	18170-L26175	Exon 27	Exon 27 4002-4003	Exon 27 4002-4003
148		18364-L23329	Exon 28	Exon 28 4196-4197	Exon 28 4196-4197
400 #		04072-L03709	Exon 29	Exon 29 4323-4324	Exon 29 4323-4324
	147	02512-L01943	Exon 30	Exon 30 4390-4391	Exon 30 4390-4391
	472	18038-L26174	Exon 31	Intron 30 3.8 kb after exon 30	Exon 31 4497-4498
166 #		02513-L01944	Exon 32	Exon 31 4534-4535	Exon 32 4597-4598
	391 #	18365-L23330	Exon 33	Exon 32 4732-4733	Exon 33 4795-4796
	166	02514-L01945	Exon 34	Exon 33 4816-4817	Exon 34 4879-4880
184 #		18367-L23332	Exon 35	Exon 34 4958-4959	Exon 35 5021-5022
	197 #	18374-L26502	Exon 36	Exon 35 12 nt before exon 35	Exon 36 12 nt before exon 36
	444 Ø	04069-L03311	OMG gene; Exon 2	OMG gene; Exon 2 NM_002544.5: 950-951,	OMG gene; Exon 2 NM_002544.5: 950-951,

Length (nt)		SALSA MLPA probe	NF1 exon based on LRG 214	NF1 exon numbering and ligation site based on NM_000267.3	NF1 exon numbering and ligation site based on NM_001042492.2
				within NF1 intron 36	within NF1 intron 36
454	∅	04075-L03310	OMG gene; Exon 2	OMG gene; Exon 2 NM_002544.5: 384-383 reverse, within NF1 intron 36	OMG gene; Exon 2 NM_002544.5: 384-383 reverse, within NF1 intron 36
214		02517-L26127	Exon 37	Exon 36 5427-5428	Exon 37 5490-5491
	211	02518-L01949	Exon 38	Exon 37 5720-5721	Exon 38 5783-5784
238		02519-L01950	Exon 39	Exon 38 6008-6009	Exon 39 6071-6072
	241	02520-L26200	Exon 40	Exon 39 6178-6179	Exon 40 6241-6242
272		02521-L22646	Exon 41	Exon 40 6390-6391	Exon 41 6453-6454
436		03853-L29796	Exon 42	Exon 41 6592-6593	Exon 42 6655-6656
	419	03854-L23156	Exon 43	Exon 42 6800-6801	Exon 43 6863-6864
	249	12021-L26199	Exon 44	Exon 43 6989-6990	Exon 44 7052-7053
	454	03856-L03307	Exon 45	Exon 44 7075-7076	Exon 45 7138-7139
	265	02522-L01953	Exon 46	Exon 45 7162-7163	Exon 46 7225-7226
289		04071-L01954	Exon 47	Exon 46 7269-7270	Exon 47 7332-7333
	300	02524-L22720	Exon 48	Exon 47 7425-7426	Exon 48 7488-7489
319		02525-L22650	Exon 49	Exon 48 7535-7536	Exon 49 7598-7599
346		02526-L01957	Exon 50	Exon 49 7671-7672	Exon 50 7734-7735
	328	13217-L22725	Exon 51	Exon 50 7799-7800	Exon 51 7862-7863
226		19363-L25737	Exon 51	Exon 50 7860-7861	Exon 51 7923-7924
373		02528-L01959	Exon 52	Exon 51 7997-7998	Exon 52 8060-8061
	154	12018-L12866	Exon 53	Exon 52 8107-8108	Exon 53 8170-8171
	307	18034-L22721	Exon 54	Exon 53 8229-8230	Exon 54 8292-8293
	345	02529-L01960	Exon 55	Exon 54 8386-8387	Exon 55 8449-8450
	382	18035-L22401	Exon 56	Exon 55 2 nt before exon 55	Exon 56 2 nt before exon 56
154		05220-L03309	Exon 57	Exon 56 8563-8564	Exon 57 8626-8627
391	«	02530-L01961	Exon 58	Exon 57 8748-8749	Exon 58 8811-8812
208	«	19361-L26126	Exon 58	Exon 57 10796-10797	Exon 58 10859-10860
136	« ¬	18363-L23328	Downstr.	Downstr. 4.8 kb after exon 57	Downstr. 4.8 kb after exon 58
			<i>stop codon</i>	<i>8838-8840 (ex 57)</i>	<i>stop codon 8901-8903 (ex 58)</i>

Probes where exon numbering between the NM sequences is different are marked in grey.

Note 1: The exon numbering and the NM_ sequences used have been retrieved on 03/2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Note 2: See page 8 and 9 for a description of the warnings (¬, Ж, #, Δ, ∅, «).