Instructions for Use

SALSA® MLPA® Probemix P044 NF2

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P044 NF2 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P044 NF2**
Version	D1
Catalogue numbers	P044-025R (25 reactions) P044-050R (50 reactions) P044-100R (100 reactions)
Basic UDI-DI	872021148P0445Q
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE CE 2797
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State in which the user and/or the patient is located.

Changes in this Product Version

As compared to version C1, twelve reference probes were replaced.

1. Intended Purpose

The SALSA MLPA Probemix P044 NF2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the *NF2* gene in genomic DNA isolated from human peripheral whole blood specimens. P044 NF2 is intended to confirm a potential cause for, and clinical diagnosis of, *NF2*-related schwannomatosis, and for molecular genetic testing of at-risk family members.

NF2-related schwannomatosis has a high incidence of mosaicism, and mosaic mutations may not be detectable in blood. Copy number variations (CNVs) detected with P044 NF2 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *NF2* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

Only in a research setting this assay can be used on DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1] Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified on page 1 of this product description. In all other countries, the product is for research use only (RUO).

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of schwannomatosis.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies *(NF2)*

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

These final ratios are only valid for germline testing.

6. Performance Characteristics

Study	Description
Expected values for copy numbers in normal and affected populations	To determine the expected values in normal and affected populations, a study using representative probemixes was conducted on over 1,500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in section 5 can be used. Cut-off values for copy number determination were verified with P044 NF2 in 37 samples from healthy individuals with normal copy number and two samples with known *NF2* CNVs. The expected FRs for the corresponding copy number were found in >99%^ of measurements in the samples tested. The use of the fixed cut-off values will not allow the detection of CNVs in all samples that are a mixture of normal and aberrant cells, such as mosaic samples.
Limit of detection	A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P044 NF2 on two samples with known *NF2* CNVs and on one sample without a normal *NF2* copy number and expected results were obtained using both the lower and upper input amount of DNA in >96%^ of measurements. Additional testing was carried out to investigate the effect of mosaicism on P044 NF2 probe performance. Mosaicism was simulated by mixing DNA samples carrying heterozygous whole gene *NF2* CNVs with varying amounts of normal DNA, creating mixtures with 30-90% CNV-positive DNA, harbouring either a heterozygous deletion or duplication. Because FR cut-off values are set using non-mosaic samples, they do not apply directly to mixed samples. In these cases, FRs depend on the level of mosaicism and may fall into an ambiguous range. For the heterozygous whole gene deletion sample, at 70% mutant DNA or higher (35% variant allele frequency (VAF)), P044 NF2 reliably detected the deletion, with the majority of probe measurements (63%^) showing FRs ≤0.80. For the heterozygous whole gene *NF2* duplication, at 60% or higher mutant DNA (30% VAF), P044 NF2 reliably detected the duplication, with the majority of probe measurements (76%^) showing FRs ≥1.20. As further evidence, Evans et al. (2020) reported that in over 1,000 patients fulfilling *NF2*-schwannomatosis criteria tested with P044 NF2, CNVs could be detected when VAF was >10%.
Interfering substances	SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction. A study using P044 NF2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA using two samples with known *NF2* CNVs and one wildtype sample. For most probes, FRs within the expected cut-off category were obtained even in the presence of EDTA, NaCl, Fe^{3+} and heparin at concentrations shown in the table below, where ambiguous results were

obtained for a small number of measurements. However, in the worst case scenario this would lead to delayed results as the assay may need to be repeated.

Hemoglobin had a more significant effect on the FRs, where both ambiguous and incorrect results were observed. Importantly, Coffalyser.Net issued warnings for hemoglobin-affected samples, which would also require the assay to be repeated.

Interferent	Source	Testing Concentration	Results* ^
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 166/171 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 163/171 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 168/171 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 167/171 measurements
Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 79/171 measurements

* Results are summarised for all probes across all three samples tested.

To minimise variability across 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. To minimise the effect of impurities in the DNA sample, see the Sample quality section under Precautions and warnings of the MLPA General Protocol.

Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and >99%^ of probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, three previously genotyped samples were tested using P044 NF2 and found to have the expected results in >98%^ of measurements. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 836/855 data points, leading to a precision of >97%^.
Clinical validity*	*NF2* mutations are found in the blood of ~40-60% of all *NF2*-related schwannomatosis patients (Evans et al. 2020, Louvrier et al. 2018, Kluwe et al. 2003), and an estimated 15-20% of mutations in *NF2* are large deletions of the promotor region, deletions/duplications of single or multiple exons, or whole gene deletions (Abo-Dalo et al. 2010, Pasmant et al. 2018, Smith et al. 2016, Wang et al. 2022, Halliday et al. 2023, GeneReviews). *Based on a 2005-2025 literature review.

^A cancer cell line with a known *NF2* CNV that was selected to assess analytical performance exhibited a consistently ambiguous FR for one NF2 probe across all experiments, leading to reduced analytical performance characteristics. The cause of this aberrant measurement has not been reported, but as this cell line is made up of four subclones, there is likely to be some variation in genotype across the subclones, and one or more of the subclones may possess a mutation at this exon.

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
22q11.21	*LZTR1*	Exon 3	11.1 kb	454	20015-L27094	¬ ∫
22q11.21	*LZTR1*	Exon 20	2.8 **M**b	400	22448-L27086	¬ ∫
22q11.23	*SMARCB1*	Exon 1	46.5 kb	433	22451-L25981	¬ ∫
22q11.23	*SMARCB1*	Exon 8	5.8 **M**b	319	08294-L20837	¬ ∫
22q12.2	*NIPSNAP1*		47.4 kb	391	02580-L02042	¬ «
22q12.2	*NF2*	Upstream	0.1 kb	143	01563-L31617	Ø
22q12.2	*NF2*	Upstream	0.8 kb	382	01581-L01135	Ø
22q12.2	*NF2*	Exon 1	32.8 kb	244	22441-L04978	
22q12.2	*NF2*	Exon 2	2.3 kb	172	01565-L31618	
22q12.2	*NF2*	Exon 3	3.1 kb	178	01566-L31619	
22q12.2	*NF2*	Exon 4	12.4 kb	197	01567-L31620	
22q12.2	*NF2*	Exon 5	1.0 kb	208	01568-L31621	
22q12.2	*NF2*	Exon 6	2.6 kb	270	22442-L02031	
22q12.2	*NF2*	Exon 7	3.0 kb	226	18696-L29634	
22q12.2	*NF2*	Exon 8	3.7 kb	254	01571-L31622	
22q12.2	*NF2*	Exon 9	3.4 kb	154	22445-L31625	
22q12.2	*NF2*	Exon 10	3.5 kb	337	22443-L31616	
22q12.2	*NF2*	Exon 11	1.4 kb	280	15774-L17826	
22q12.2	*NF2*	Exon 12	1.5 kb	310	01575-L31623	
22q12.2	*NF2*	Exon 13	3.4 kb	158	22439-L31614	
22q12.2	*NF2*	Exon 14	3.2 kb	328	01577-L01149	
22q12.2	*NF2*	Exon 15	2.0 kb	190	22440-L31615	
22q12.2	*NF2*	Intron 15	11.3 kb	355	03318-L02736	Ø
22q12.2	*NF2*	Exon 16	0.1 kb	136	22444-L31624	
22q12.2	*NF2*	Exon 16	3.3 kb	366	01580-L29633	
22q12.2	*NF2*	Exon 16	30.9 kb	217	22446-L31626	
22q12.2	*CABP7*		3.7 **M**b	409	03317-L31857	¬ «
22q12.3	*LARGE1*		7.8 **M**b	463	12460-L13461	¬
22q13.2	*EP300*		8.8 **M**b	292	22449-L31628	¬
22q13.33	*ALG12*			427	22450-L31629	¬
2p22	Reference - *SPAST*			299	07127-L06736	*
2p13	Reference - *DYSF*			183	08819-L08879	*
3q13	Reference - *CASR*			166	05705-L05223	*
8p23	Reference - *CTSB*			130	01212-L00766	*
8p21	Reference - *NEFL*			476	14114-L19228	*
8q13	Reference - *EYA1*			234	20829-L28844	*
10p11	Reference - *ZNF25*			346	06214-L06020	*
11p13	Reference - *PAX6*			418	07081-L06710	*
11q12	Reference - *SERPING1*			202	08187-L08081	*
12q12	Reference - *LRRK2*			373	04278-L03682	*
12q14	Reference - *HMGA2*			149	15075-L30701	*
13q12	Reference - *GJB6*			259	22189-L31241	*
21q11	Reference - *HSPA13*			445	05916-L14204	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *NF2*, *LZTR1* and *SMARCB1* exon numbers are derived from MANE project and are based on the MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Chromosomal bands are based on: hg18

7. Precautions and Warnings

Probe changes

*	New probes.

Probe warnings

¬	This probe is a flanking probe, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probes are unlikely to be related to the condition tested, unless indicated otherwise (see warning for probes with symbol ∫).
«	These probes are 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.
Ø	These probes target a sequence outside of the exonic sequence of the reference transcript. Copy number alterations of only these probes are of unknown clinical significance.
∫	*SMARCB1* and *LZTR1* are tumour suppressor genes associated with schwannomatosis, which is a tumour predisposition syndrome related to *NF2*-related schwannomatosis. Additionally, loss of heterozygosity (LOH) of *NF2* in sporadic and *NF2*-related schwannomatosis-associated tumours often includes loss of *SMARCB1* and *LZTR1*. These probes also help to determine the extent of the deletion/duplication on chromosome 22.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No

1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. Large deletions are often detected with this probemix. The slope correction algorithm in Coffalyser.Net may confuse a large deletion for sloping, leading to an incorrectly applied slope correction and a false warning or ambiguous results for multiple probes. Incorrectly applied slope correction can cause an FSLP warning in Coffalyser.Net or ambiguous results for multiple probes. If you suspect that slope correction was incorrectly applied, we recommend contacting info@mrcholland.com for assistance.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations
1. MLPA analysis provides information on the average situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected in germline samples in cases of mosaicism, or in tumour samples if the percentage of tumour cells is low. In addition, subclonality of the aberration in tumour samples affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes do show a copy number alteration in a patient sample, especially in tumours with high chromosomal instability.
2. *NF2*-related schwannomatosis has a high incidence of mosaicism, and final ratios are affected by the proportion of affected cells in the tissue sample from which the DNA was purified. The use of the fixed cut-off values as mentioned in the table under section 5 will not allow the detection of deletions/duplications in all samples that are a mixture of normal and abnormal cells, such as mosaic samples. In case several probes targeting adjacent sequences have an unusual value, but do not reach the usual threshold values for a deletion/duplication, mosaicism is a possible cause.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Abo-Dalo B et al. (2010). Large intragenic deletions of the NF2 gene: breakpoints and associated phenotypes. *Genes Chromosomes Cancer*. 49(2):171-175.
2. Evans DG et al. (2020). Incidence of mosaicism in 1055 de novo NF2 cases: much higher than previous estimates with high utility of next-generation sequencing. *Genet Med*. 22(1):53-59.
3. Halliday D et al. (2023). Updated protocol for genetic testing, screening and clinical management of individuals at risk of NF2-related schwannomatosis. *Clin Genet*. 103(5):540-552.
4. Kluwe L et al. (2003). Molecular study of frequency of mosaicism in neurofibromatosis 2 patients with bilateral vestibular schwannomas. *J Med Genet*. 40(2):109-114.
5. Louvrier C et al. (2018). Targeted next-generation sequencing for differential diagnosis of neurofibromatosis type 2, schwannomatosis, and meningiomatosis. *Neuro Oncol*. 20(7):917-929.
6. Pasmant E et al. (2018). Neurofibromatosis type 2 French cohort analysis using a comprehensive NF2 molecular diagnostic strategy. *Neurochirurgie*. 64(5):335-341.
7. Smith MJ et al. (2016). The contribution of whole gene deletions and large rearrangements to the mutation spectrum in inherited tumor predisposing syndromes. *Hum Mutat*. 37(3):250-256.
8. Wang VY et al. (2022). Clinical manifestations and genetic analysis of a family with neurofibromatosis type 2. *Acta Otolaryngol*. 142(1):36-42.

Implemented changes in the product description
Version D1-01 – 11 June 2026 (03S) - Changes in this product version compared to the C1 version added. - Notified body number added to CE mark. - Basic UDI-DI added. - Intended purpose updated to remove the proportion of *NF2*-related schwannomatosis patients who exhibit mosaicism. - Performance characteristics table added. - Reference to SSP added. - Probe contents table updated for new probemix version. - Only exon numbering using the MANE Select transcript is now included in the probe content table. - Salt sensitivity probe warnings added for NIPSNAP1 probe 02580-L02042 and CABP7 probe 03317-L31857. - The warning regarding probes outside of the known coding region reworded for accuracy. - Limitation added regarding the use of cut-off values in mosaic samples. - Probemix is now IVDR certified.