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	C1
Catalogue numbers	P044-025R (25 reactions) P044-050R (50 reactions) P044-100R (100 reactions)
Basic UDI-DI	n.a.
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
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

Compared to the B3 version

Nine new target probes have been included, one replaced, and one removed. Ten reference probes have been replaced and the total number of reference probes has been increased to 13. Several probes have changed in length but not in the sequence detected.

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 (~15% of patients are mosaic) 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

In ~70% of patients with a clinical diagnosis of *NF2*-related schwannomatosis, a germline mutation in the *NF2* gene is identified, and 15-20% of all known pathogenic *NF2* mutations are large deletions or duplications. Therefore, the minimal diagnostic sensitivity of P044-C1 is estimated at 10-15%. Analytical performance for the detection of deletions/duplications in *NF2* is very high and can be considered >99% (based on a 2005-2024 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms 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.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warning
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 (Exon 16)	11.3 kb	355	03318-L02736	Ø
22q12.2	*NF2*	Exon 16 (17)	0.1 kb	136	22444-L31624	
22q12.2	*NF2*	Exon 16 (17)	3.3 kb	366	01580-L29633	
22q12.2	*NF2*	Exon 16 (17)	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	¬
2p13	Reference - *DYSF*			263	08812-L24457	
2q13	Reference - *EDAR*			148	14199-L23450	
3q11	Reference - *CPOX*			472	14846-L16554	
4p13	Reference - *ATP8A1*			236	19652-L26684	
4q22	Reference - *PKD2*			416	22471-L24662	
5q31	Reference - *IL4*			129	18709-L26847	
6p12	Reference - *PKHD1*			374	10718-L31856	
9q22	Reference - *ROR2*			184	16915-L19859	
12p13	Reference - *GRIN2B*			301	17452-L21208	
15q21	Reference - *FBN1*			346	04337-L20895	
16p11	Reference - *TGFB1I1*			203	13121-L31675	
19p13	Reference - *RNASEH2A*			166	16253-L31432	
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. Annotation of one probe with a target at the edge of or slightly outside the coding region, is altered. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18

7. Precautions and Warnings

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 ∫).
Ø	These probes target a sequence outside of the known coding region. 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.

Technique-specific limitations
See the MLPA General Protocol.

Implemented changes in the product description
Version C1-05 – 05 March 2025 (03S) - Product description updated to a new template. - Exon numbering updated for *NF2*, *LZTR1* and *SMARCB1* probes. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Probe warning symbols for probes 02580-L02042 and 03317-L31857 being located in a GC-rich region removed. - SNV rs121434259/COSM21991 may affect the probe signal. However, the warnings for this SNV present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.