Instructions for Use

SALSA® MLPA® Probemix P461 STRC-CATSPER2-OTOA

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 P461 STRC-CATSPER2-OTOA product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P461 STRC-CATSPER2-OTOA**
Version	B1
Catalogue numbers	P461-025R (25 reactions) P461-050R (50 reactions) P461-100R (100 reactions)
Basic UDI-DI	872021148P4616C
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797
RUO	ALL OTHER COUNTRIES

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

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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.

More Information: www.mrcholland.com	
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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 or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version A1, new target probes, amongst others probes for *STRCP1*, have been added, and several target and flanking probes have been removed. Most reference probes have been replaced.

1. Intended Purpose

The SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] to be used with genomic DNA isolated from human peripheral blood. The probemix is intended for the detection of deletions in the *STRC* gene, as well as gene conversions between *STRC* and its pseudogene *STRCP1* to confirm a potential cause for and establish a clinical diagnosis of Autosomal recessive deafness 16 (DFNB16). Moreover, this assay can detect deletions of *STRC* and *CATSPER2* genes, to confirm a potential cause for and establish a clinical diagnosis of *STRC*-related autosomal recessive hearing loss, including Deafness-infertility syndrome (DIS). Lastly, P461 STRC-CATSPER2-OTOA allows for the detection of deletions in the *OTOA* gene to confirm a potential cause for and establish a clinical diagnosis of Autosomal recessive deafness 22 (DFNB22). The probemix can also be used for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P461 STRC-CATSPER2-OTOA should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Numerous defects in the *STRC* and *OTOA* genes 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, parental evaluation, 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, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[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 DFNB22 or *STRC*-related autosomal recessive hearing loss, including DIS and DFNB16.	
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.
Validation Samples (Required)	• This probemix contains target probes that bind to sequences with natural variation. The validation experiments of this probemix should result in a standard deviation ≤0.10 for all reference probes.	

*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 (*STRC*/*CATSPER2*/*OTOA*)

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.

Due to the high sequence similarity between the *STRC* gene and its pseudogene *STRCP1*, a small background signal can be visible for the STRC exon 19 probe at 311 nt when no *STRC* exon 19 is present and a final ratio of 0.0 is expected. This background signal might be displayed as an intra ratio percentage instead of a final ratio (see our support portal for more details).

A detailed interpretation guide for gene conversions and examples of complex gene testing of families can be found on the P461 product page on www.mrcholland.com.

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 was conducted on over 1500 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 the section above can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA in 101 samples from healthy individuals with normal copy numbers and 15 samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.
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 recommended input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA on four samples with known CNVs and on one sample without any aberration. In three of the positive samples, one measurement gave an ambiguous result, however since all target probes need to be evaluated together, the expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl, EDTA and hemoglobin) can affect the MLPA reaction. A study using SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on four samples with known CNVs and on one sample without any aberration. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 316/390 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 365/390 measurements

	Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 374/390 measurements
	Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 375/390 measurements
	Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 276/390 measurements
	* Results are summarised for all probes across all five samples tested. Hemoglobin and EDTA had the largest effect on copy number determination, whereas a milder effect was observed with NaCl, $FeCl_3$ and Heparin. While the latter led to ambiguous ratios and potentially delayed results, Hemoglobin and EDTA caused incorrect ratios and consequently false results. 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.			
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 all probes met the quality criteria for specificity.			
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, 14 previously genotyped samples were tested using SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple laboratories. Results showed a correct call in 514/518 data points, leading to a precision of >99%.			
Clinical validity*	Deafness-infertility syndrome (DIS): All cases of DIS are caused by homozygous deletions of the whole *STRC* and *CATSPER2* genes (GeneReviews). DFNB16: It is estimated that 40% of individuals with *STRC*-related Hearing Loss carry a biallelic contiguous gene deletion involving *STRC* and *CATSPER2*, causative for DIS (Nishio and Usami 2022). Additionally, 30% of individuals with DFNB16 are compound heterozygous for one *STRC* pathogenic variant and one contiguous gene deletion involving *STRC* and *CATSPER2*. The remaining 30% of individuals with DFNB16 carry biallelic *STRC* pathogenic variants, of which most are deletions (Redfield and Shearer 2023). DFNB22: *OTOA* is the gene most commonly implicated in DFNB22 due to copy number variants (CNVs). While the overall prevalence of *OTOA*-related CNVs in the general population is not well established, two independent studies in hearing loss cohorts have reported that CNVs in *OTOA* contribute to approximately 0.3% to 8% of DFNB22 cases (Shearer et al. 2014; Sugiyama et al. 2019). *(Based on a 2021-2025 literature review)			

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 pairs	Probe number	Warnings
15q15.3	*PPIP5K1*		40.1 kb	220		20151-L27407	¬ #
15q15.3	*CKMT1B*		0.9 kb	190	A	20147-L31814	¬ ¥ #
15q15.3	*STRC*	Exon 28	0.8 kb	187	B	22755-L32078	* Ж #
15q15.3	*STRC*	Exon 25	0.6 kb	226		22602-L31815	* + #
15q15.3	*STRC*	Exon 24	1.8 kb	166		20146-L27402	#
15q15.3	*STRC*	Exon 23	1.5 kb	380	D	22603-L31816	* #
15q15.3	*STRC*	Exon 20	0.4 kb	472	E	22604-L31817	* Ж #
15q15.3	*STRC*	Intron 19 (Exon 19)	0.2 kb	244		20155-L32077	Ø ¥ #
15q15.3	*STRC*	Exon 19	33.6 kb	311	C	22754-L32091	* Ж # ◊
15q15.3	*CATSPER2*	Exon 7	8.0 kb	297		20160-L28719	#
15q15.3	*CATSPER2*	Exon 4	0.8 kb	483		22592-L31804	* #
15q15.3	*CATSPER2*	Exon 2	0.8 kb	303		20236-L28905	+ #
15q15.3	*CATSPER2*	Exon 1	0.2 kb	444		22606-L31819	* #
15q15.3	*CATSPER2*	Exon 1	50.1 kb	274		22593-L32079	*
15q15.3	*CKMT1A*		0.9 kb	149	A	22682-L31933	* ¬ Ж #
15q15.3	*STRCP1*	Exon 28	2.9 kb	388	B	22684-L31935	* Ж # ◎
15q15.3	*STRCP1*	Exon 23	1.4 kb	256	D	22607-L31820	* Ж # ◎
15q15.3	*STRCP1*	Exon 20	0.7 kb	364	E	22608-L31821	* Ж # ◎
15q15.3	*STRCP1*	Exon 19	41.8 kb	208	C	22753-L32338	* Ж # ◎
15q15.3	*PDIA3*			263		20157-L28761	¬ #
16p12.2	*METTL9*	Exon 2	12.3 kb	290		20588-L28718	¬
16p12.2	*METTL9*	Exon 4	53.5 kb	433		20600-L28841	¬
16p12.2	*OTOA*	Exon 2	0.6 kb	155		20584-L32088	¥
16p12.2	*OTOA*	Exon 5	6.1 kb	407		23046-L28256	¥
16p12.2	*OTOA*	Exon 7	2.1 kb	143		22686-L28243	¥
16p12.2	*OTOA*	Exon 8	13.6 kb	346		22595-L31807	*
16p12.2	*OTOA*	Exon 11	4.3 kb	418		20599-L28257	
16p12.2	*OTOA*	Exon 12	13.9 kb	373		22596-L31808	*
16p12.2	*OTOA*	Exon 16	0.3 kb	337		20590-L28248	
16p12.2	*OTOA*	Exon 17	3.5 kb	232		20586-L28244	
16p12.2	*OTOA*	Exon 18	5.3 kb	202		22597-L31809	*
16p12.2	*OTOA*	Exon 20	229.3 kb	454		22598-L31810	*
16p12.1	*UQCRC2*	Exon 3 (Exon 4)	16.4 kb	136		20583-L28241	¬ +
16p12.1	*UQCRC2*	Exon 11		328		23045-L28255	¬ ¥
2q	Reference			319		06580-L30872	*
4p	Reference			490		20096-L27538	*
4p	Reference			124		19616-L26275	*
6p	Reference			214		10698-L11280	*
6q	Reference			352		13400-L20982	*
7p	Reference			177		04359-L03779	*
8q	Reference			463		10108-L10532	*
10q	Reference			160		09267-L09527	*
12p	Reference			394		03178-L18979	
15q	Reference			250		15065-L16823	*
21q	Reference			267		19015-L32525	*

As rearrangements or gene conversions can occur, probe pairs were designed for a number of *STRC* and *STRCP1* exons (19, 20, 23 and 28; see Probe pairs column). To facilitate interpretation of results, *STRCP1* exon numbering is adjusted to match the *STRC* exon numbering.
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 exon numbers for *STRC*, *STRCP1*, *CATSPER2*, *OTOA, METTL9 and UQCRC2* are derived from MANE project and are based on 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 changes

*	New probes in the B1 version.
¥	Probes changed in this product version. Minor alteration, no change in sequence detected.

Probe warnings

¬	These probes are flanking probes, 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.
◊	Due to the high sequence similarity between the *STRC* gene and its pseudogene *STRCP1*, a small background signal can be visible for the STRC exon 19 probe at 311 nt when no *STRC* exon 19 is present and a final ratio of 0.0 is expected.
Ж	These probes are sensitive to sample DNA depurination. Use of depurinated DNA will lead to a lower signal of these probes. Depurination can occur in acidic conditions, e.g. due to insufficient buffering capacity during sample DNA denaturation. When this occurs only in reference samples, it will result in an increased ratio in the test samples.

Ø	This probe targets a sequence outside of the known coding region.
#	The specificity of these probes relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only these probes can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
+	The ligation site of this/these probe(s) is >20 nt away from the nearest exon.
๑	The clinical significance of *STRCP1* probe aberrations is not known. Probes for *STRCP1* are included as an aid to differentiate between *STRC* CNVs and possible gene conversions.

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. Before testing patient samples, testing of healthy individuals is required to identify suitable reference samples for proper data analysis.
6. Gene conversion between *STRC* and *STRCP1* can occur at several sites. One example of a clinical significant gene conversion is a nonsense mutation in exon 20 from *STRCP1* to *STRC*, leading to a stop codon in *STRC* (Vona et al. 2015).
7. As rearrangements or gene conversions can occur, probe pairs were designed for a number of *STRC* and *STRCP1* exons (19, 20, 23 and 28; see Table with Content for probe pairs). To facilitate interpretation of results, *STRCP1* exon numbering is adjusted to match the *STRC* exon numbering. In a normal situation a probe pair has a combined final ration of 2 (*STRC* FR of 1.0 and *STRCP1* FR of 1.0). In case of a gene conversion a probe pair can still have a combined final ratio of 2, but different combinations are possible, such as *STRC* FR of 0.5 and *STRCP1* FR of 1.5. However, the combined final ratio might also be higher or lower than 2 if there is an additional deletion or duplication present in the gene or pseudogene. Examples for gene conversions can be found on the P461 product page.
8. Interpretation of complex results may require parental analysis to ensure accuracy. In rare cases, analysis of grandparental samples can provide additional clarity and improve the understanding of the proband's genetic profile.

Technique-specific precautions

See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The *STRC*, *CATSPER2* and *OTOA* genes have highly homologous pseudogenes. In rare cases, apparent duplications might therefore be due to sequence changes in the homologous genes.
2. Certain exons of the *STRC*, *CATSPER2*, and *OTOA* genes are not covered due to the absence of corresponding probes.

Technique-specific limitations

See the MLPA General Protocol.

9. References Cited in this IFU

1. Nishio SY and Usami SI. (2022). Frequency of the STRC-CATSPER2 deletion in STRC-associated hearing loss patients. *Sci Rep*. 12:634.
2. Redfield S and Shearer AE. (2023). STRC-Related Autosomal Recessive Hearing Loss. In M. P. Adam, J. Feldman, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. W. Gripp, & A. Amemiya (Eds.), *GeneReviews((R))*. Seattle (WA).
3. Shearer AE et al. (2014). Copy number variants are a common cause of non-syndromic hearing loss. *Genome Med*. 6:37.
4. Sugiyama K et al. (2019). Mid-Frequency Hearing Loss Is Characteristic Clinical Feature of OTOA-Associated Hearing Loss. *Genes (Basel)*. 10.
5. Vona B et al. (2015). DFNB16 is a frequent cause of congenital hearing impairment: implementation of STRC mutation analysis in routine diagnostics. *Clin Genet*. 87:49-55.

Implemented changes in the product description

Version B1-04 – 22 January 2026 (03S)

- The product description has been updated to a new template.
- Intended purpose updated to specify that assay is manual and detection of duplications for *STRC*, *CATSPER2* and *OTOA* removed.
- Warning for a ligation site >20 nt away from the nearest exon added to STRC probe 22602-L31815, CATSPER2 probe 20236-L28905 and UQCRC2 probe 20583-L28241.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Warning for a probe targeting a sequence outside of the known coding region included for STRC probe 20155-L32077.
- Exon numbering updated for *UQCRC2* probe 20583-L28241 according to MANE.
- Probe warning for single nucleotide differences compared to a related gene or pseudogene added for probes 20147-L31814, 22755-L32078, 22602-L31815, 22603-L31816, 22604-L31817, 22754-L32091,22682-L31933, 22684-L31935, 22607-L31820, 22608-L31821 and 22753-L32338.
- New probe warning ◊ for STRC probe 22754-L32091 added.
- SNVs rs141809944, rs188647272, rs551423552 and rs2614832 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Probemix is now IVDR certified.