Instructions for Use
SALSA® MLPA® Probemix P114 Long-QT

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 P114 Long-QT product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P114 Long-QT**
Version	C1
Catalogue numbers	P114-025R (25 reactions) P114-050R (50 reactions) P114-100R (100 reactions)
Basic UDI-DI	872021148P1145L
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 or country in which the user and/or the patient is located.

Changes in this Product Version

Compared to version B3, three new target probes for *KCNJ2* and one new target probe for *KCNQ1* exon 6 were included, one target probe was replaced, and four target probes were adjusted. Six reference probes were replaced and one reference probe length was changed.

1. Intended Purpose

The SALSA MLPA Probemix P114 Long-QT 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 *KCNQ1* and *KCNH2* genes in genomic DNA obtained from human peripheral whole blood specimens[3]. P114 Long-QT is intended to confirm a potential cause for and clinical diagnosis of congenital Long-QT syndrome (LQTS) types 1 and 2 or Jervell Lange-Nielsen syndrome (JLNS); a recessive form of LQTS associated with homozygous or compound heterozygous mutations in *KCNQ1*. P114 Long-QT can also be used for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P114 Long-QT 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 in *KCNQ1* and *KCNH2* 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, 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, newborn or population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations. This assay is not for use with DNA extracted from dried blood spots.

[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.

[3] Certain probes targeting additional genes included in P114 Long-QT may only be used in a research setting. The following table summarises which probes are for IVD use or exclusively restricted to be used in a research setting:

IVD Targets	RUO Targets
KCNQ1, KCNH2	*KCNE1, KCNE2, KCNJ2*

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 LQTS or JLNS.	
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 (*KCNQ1/KCNH2/KCNE1/KCNE2/KCNJ2*)

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.

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy numbers in normal and affected populations</td><td>To determine the expected values in normal and affected populations, a study was conducted on over 1500 MLPA reactions and samples with and without abnormal copy numbers. When the standard deviation of each probe over all the reference samples is ≤0.10, the FRs stated in the tables above can be used. Cut-off values were verified with SALSA MLPA Probemix P114 Long-QT in 45 samples from healthy individuals with normal KCNQ1 and KCNH2 copy numbers, and five samples with known KCNQ1 or KCNH2 CNVs. The expected FRs for the corresponding copy number were found in all samples tested.</td></tr>
<tr><td>Limit of detection</td><td>A study that evaluated the acceptable minimum and maximum amount of sample DNA revealed that the use of 50-250 ng of human DNA is the recommended input. 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 P114 Long-QT on three samples with known KCNQ1 or KCNH2 CNVs, and one sample with a normal KCNQ1 and KCNH2 copy number. The expected results were obtained in 97.5% of cases.</td></tr>
<tr><td>Interfering substances</td><td>Impurities in the DNA sample can affect the MLPA reaction. To minimise this effect, see Sample quality section under Precautions and warnings of the MLPA General Protocol. A study using SALSA MLPA Probemix P114 Long-QT was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on three samples with known KCNQ1 or KCNH2 CNVs, and one sample with normal KCNQ1 and KCNH2 copy number. The interferents tested, their source, their testing concentrations and results are presented in the table below.
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Expected FR for 318/340 measurements**</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Expected FR for 398/408 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Expected FR for 403/408 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Expected FR for 391/408 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µL</td><td>Expected FR for 0/408 measurements</td></tr>
<tr><td>Blank (TE)</td><td>-</td><td>-</td><td>Expected FR for 394/408 measurements</td></tr>
</table>
* Results summarised for 18 KCNQ1 and 16 KCNH2 probes across four samples in triplicate.
** Results summarised across two samples in triplicate and two samples in duplicate.
Hemoglobin had the largest effect on copy number determination: FRs outside the cut-off values were obtained for all replicates. In the presence of hemoglobin, Coffalyser.Net warnings were obtained in all samples. In the presence of EDTA, NaCl, Fe^{3+} and heparin, a few measurements were outside the cut-off values (6.4%, 2.4%, 1.2% and 4.2% respectively), and mostly within the ambiguous range. Coffalyser.Net warnings were obtained in all deviating measurements for EDTA, NaCl and Fe^{3+}. In the absence of interferents, 3.4% of measurements were outside the cut-off values.
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.</td></tr>
<tr><td>Cross-reactivity</td><td>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. Experiments on five samples with known KCNQ1 or KCNH2 CNVs and 45 samples with normal KCNQ1 and KCNH2 copy numbers were performed to determine whether probes are specific to their target sequence. The expected FRs were obtained in >99% of measurements.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, five previously genotyped samples were tested using SALSA MLPA Probemix P114 Long-QT and found to have the expected results in >99% of measurements. Precision was tested by repeatedly testing three samples with known KCNQ1 or KCNH2 CNVs, and one sample with normal KCNQ1 and KCNH2 copy number, over multiple days, and by multiple operators. Results showed a correct call in 98% of measurements.</td></tr>
<tr><td>Clinical validity*</td><td>LQTS is mostly due to point mutations in KCNQ1 (30-35% of cases) and KCNH2 (25-30% of cases). CNVs in these genes have been identified in 3% of individuals with LQTS, after mutation-negative results from sequencing.

*Based on a 2006-2024 literature review</td></tr>
</table>

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
7q36.1	*KCNH2*	Exon 15 (Exon 16)	0.1 kb	328	15851-L18358	
7q36.1	*KCNH2*	Exon 15 (Exon 16)	1.5 kb	165	15850-L17933	
7q36.1	*KCNH2*	Exon 14 (Exon 15)	0.6 kb	427	04403-L19825	
7q36.1	*KCNH2*	Exon 12 (Exon 13)	0.9 kb	244	15849-L17932	
7q36.1	*KCNH2*	Exon 11 (Exon 12)	0.5 kb	472	15848-L17931	
7q36.1	*KCNH2*	Exon 10 (Exon 11)	1.2 kb	346	04097-L20370	
7q36.1	*KCNH2*	Exon 9 (Exon 10a)	0.7 kb	238	03532-L02898	
7q36.1	*KCNH2*	Exon 8 (Exon 9)	0.7 kb	215	15847-L31833	#
7q36.1	*KCNH2*	Exon 7 (Exon 8)	0.9 kb	226	15846-L29772	
7q36.1	*KCNH2*	Exon 6 (Exon 7)	2.7 kb	178	03531-L02897	#
7q36.1	*KCNH2*	Intron 5 (Exon 6)	2.2 kb	490	22119-L18503	Ø
7q36.1	*KCNH2*	Exon 5	0.9 kb	263	15845-L29770	
7q36.1	*KCNH2*	Exon 4	1.4 kb	391	04099-L25306	
7q36.1	*KCNH2*	Exon 3	15.0 kb	274	03528-L02894	
7q36.1	*KCNH2*	Exon 2	3.1 kb	209	22361-L31523	
7q36.1	*KCNH2*	Exon 1		154	03526-L02892	
11p15.5	*KCNQ1*	Exon 1	16.2 kb	142	03535-L02901	
11p15.5	*KCNQ1*	Intron 1 (Exon 2)	66.3 kb	436	03537-L02903	Ø
11p15.5	*KCNQ1*	Exon 2 (Exon 3)	42.7 kb	220	03539-L02905	
11p15.5	*KCNQ1*	Exon 3 (Exon 4)	0.6 kb	256	03540-L18356	
11p15.5	*KCNQ1*	Exon 4 (Exon 5)	0.7 kb	294	22163-L31834	
11p15.5	*KCNQ1*	Exon 5 (Exon 6)	0.8 kb	199	22117-L31489	Δ
11p15.5	*KCNQ1*	Exon 6 (Exon 7)	10.6 kb	337	03542-L20369	
11p15.5	*KCNQ1*	Exon 7 (Exon 8)	1.7 kb	364	16248-L19751	
11p15.5	*KCNQ1*	Exon 8 (Exon 9)	2.4 kb	409	03544-L19823	
11p15.5	*KCNQ1*	Exon 9 (Exon 10)	1.2 kb	148	03545-L04801	
11p15.5	*KCNQ1*	Exon 10 (Exon 11)	73.2 kb	171	16243-L18501	
11p15.5	*KCNQ1*	Exon 11 (Exon 12)	106.9 kb	310	22164-L31192	
11p15.5	*KCNQ1*	Exon 12 (Exon 13)	7.2 kb	268	03550-L18357	
11p15.5	*KCNQ1*	Exon 13 (Exon 14)	1.0 kb	301	03551-L02917	
11p15.5	*KCNQ1*	Exon 14 (Exon 15)	1.0 kb	373	14793-L16504	
11p15.5	*KCNQ1*	Exon 15 (Exon 16)	69.9 kb	382	14064-L18359	
11p15.4	*KCNQ1*	Exon 16 (Exon 17)	0.9 kb	454	03554-L02920	
11p15.4	*KCNQ1*	Exon 16 (Exon 17)		418	03555-L29768	
17q24.3	*KCNJ2*	Exon 1	5.6 kb	190	13979-L31068	
17q24.3	*KCNJ2*	Exon 2	0.8 kb	131	22089-L15549	
17q24.3	*KCNJ2*	Exon 2		481	22090-L31320	#
21q22.11	*KCNE2*	Exon 1	6.5 kb	184	05072-L04472	
21q22.11	*KCNE2*	Exon 2	78.8 kb	282	05073-L04473	
21q22.12	*KCNE1*	Exon 4	0.2 kb	463	05071-L04471	
21q22.12	*KCNE1*	Exon 4	9.0 kb	136	05070-L04470	
21q22.12	*KCNE1*	Exon 3 (Exon 2)	52.5 kb	319	15854-L17947	
21q22.12	*KCNE1*	Exon 2 (Exon 1)		250	16247-L18505	
2p	Reference			288	15880-L30312	
4p	Reference			500	19675-L27812	
5q	Reference			124	18709-L21056	
6p	Reference			202	10697-L31832	
8q	Reference			160	15974-L18507	
10p	Reference			400	01237-L00568	
12p	Reference			355	11614-L12374	
13q	Reference			445	16286-L31835	
18q	Reference			232	16428-L25931	

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 *KCNQ1, KCNH2, KCNE1, KCNE2* and *KCNJ2* exon numbers 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. 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

Δ	This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
Ø	This/these probe(s) target(s) (a) sequence(s) 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.

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. Deletions/duplications spanning both whole *KCNE1* and *KCNE2* genes have been found in individuals not affected with LQTS (Williams et al. 2015).
6. The presence of hemoglobin was shown to strongly affect probe signal. Coffalyser.Net will issue warnings for affected samples.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. Target probes for *KCNE1, KCNE2* and *KCNJ2* CNVs are included to be used for research purposes only and not for diagnostic use.
2. No probes are present for *KCNH2* exon 13 and *KCNE1* exon 1.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Williams VS et al. (2015). Multiplex ligation-dependent probe amplification copy number variant analysis in patients with acquired long QT syndrome. Europace. 17:635-641.

Implemented changes in the product description

Version C1-05 – 25 September 2025 (03S)

- Product description was updated to a new template.
- Intended purpose was updated specifying the assay is manual. *KCNE1*, *KCNE2* and *KCNJ2* genes removed from the Intended purpose.
- The probes targeting *KCNE1*, *KCNE2* and *KCNJ2* are no longer intended for diagnostic use.
- Exon numbering updated for *KCNQ1*, *KCNH2* and *KCNE1*.
- Warnings for targets outside of the coding region added for probes 22119-L18503 and 03537-L02903.
- Warnings for probes 05071-L04471, 05070-L04470 and 15854-L17947 were removed due to lack of confirmed evidence for the presence of the *KCNE1b* pseudogene in the human genome. Experiments performed at MRC Holland on a duplication positive sample suggest that *KCNE1b* is not present in the human genome.
- Probemix-specific precaution on sensitivity to hemoglobin added.
- Probemix-specific limitation on probes not covering all *KCNH2* and *KCNE1* exons added.
- Probemix is now IVDR certified.