Product Description
SALSA® MLPA® Probemix P443-A2 KANSL1

To be used with the MLPA General Protocol.

Version A2
As compared to version A1, three reference probes have been replaced and one reference probe has been removed. For complete product history see page 7.

Catalogue numbers:
- **P443-025R:** SALSA MLPA Probemix P443 KANSL1, 25 reactions.
- **P443-050R:** SALSA MLPA Probemix P443 KANSL1, 50 reactions.
- **P443-100R:** SALSA MLPA Probemix P443 KANSL1, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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.

General information
The SALSA MLPA Probemix P443 KANSL1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *KANSL1* gene, which is associated with Koolen-de Vries syndrome.

Koolen-de Vries syndrome, also known as 17q21.31 deletion syndrome, is characterised by developmental delay, neonatal/childhood hypotonia, dysmorphisms, congenital malformations and behavioural features. Global psychomotor developmental delay is noted in all individuals from an early age. The majority of individuals with the 17q21.31 deletion syndrome function in the mild to moderate range of intellectual disability. Other findings include epilepsy, congenital heart defects, renal and urologic anomalies and cryptorchidism. *KANSL1* (previously known as *KIAA1267*) is located in the 17q21.31 critically deleted region (Zollino et al. 2012). Please note, that 17q21.31 duplications affecting the 5' coding exons of the *KANSL1* gene have been described in healthy individuals by Steinberg et al. (2012). The authors found that almost 60% of Europeans carry at least one of three known duplications. In our quality testing we found comparable results.

The *KANSL1* gene (15 exons) spans ~163 kb of genomic DNA and is located on chromosome 17q21.31, ~41 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK24676/.

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *KANSL1* exon numbering used in this P443-A2 KANSL1 product description is the exon numbering from the NG_032784.1 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P443-A2 KANSL1 contains 32 MLPA probes with amplification products between 128 and 364 nucleotides (nt). This includes 19 probes for the *KANSL1* gene: probes for each exon except exon 12 and extra probes for exons 2, 3, 4 and 15. This probemix also contains one extra probe for the first exon of transcript variant 2 (NM_015443.4 sequence), which is located 0.5 kb upstream of exon 1 of the main transcript variant 1 (NM_001193466.2 sequence). Furthermore, this probemix contains four centromeric flanking probes for the *KANSL1* gene. Two are located in the *MAPT* gene and two in the *CRHR1* gene. In addition, nine reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains 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, and 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.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal 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.mrcholland.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 reference probes over the experiment.

Required specimens
Extracted DNA, 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
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. 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 different unrelated individuals who are from families without a history of Koolen-de Vries syndrome. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis

Coffalyser.Net software should 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.mrcholland.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 standard deviation of each individual reference probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *KANSL1* 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 (as described for *DMD* by 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 not result in inactivation of that gene copy.

- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P443 specific note:

- Duplications of the 5' coding exons of the *KANSL1* gene have been described in a large portion of healthy individuals!

Limitations of the procedure

- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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 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.

***KANSL1* mutation database**

https://databases.lovd.nl/shared/genes/KANSL1. We strongly encourage users to deposit positive results in the Leiden Open Variation 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 SNVs and unusual results (e.g., a duplication of *KANSL1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P443-A2 KANSL1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*KANSL1*	17q21.31
64-105	Control fragments – see table in probemix content section for more information			
128	Reference probe 00797-L00093	5q		
133 «	**KANSL1** probe S0991-L26452		**Exon 1**	
139	**KANSL1** probe S0992-L26451		**Exon 4**	
144	**KANSL1** probe S0993-L26450		**Exon 3**	
148	Reference probe 17032-L20095	10p		
154	**KANSL1** probe 19200-L25250		**Exon 6**	
160 ¬	CRHR1 probe 08369-L14410			centromeric
166	**KANSL1** probe 19201-L25921		**Exon 2**	
173	**KANSL1** probe 19202-L25252		**Exon 5**	
181 Ж	**KANSL1** probe 19203-SP0769-L25733		**Exon 14**	
188 Ж	**KANSL1** probe 19204-SP0770-L25254		**Exon 8**	
202 ¬	MAPT probe 08353-L08206			centromeric
211	**KANSL1** probe 19206-L25256		**Exon 15**	
219	**KANSL1** probe 19207-L25257		**Exon 7**	
225 *	Reference probe 19059-L24946	5p		
232 ¬	CRHR1 probe 08367-L25922			centromeric
238	**KANSL1** probe 19208-L25258		**Exon 2**	
244 *	Reference probe 18664-L24018	11p		
252	**KANSL1** probe 19209-L25766		**Exon 11**	
274 Ж	**KANSL1** probe 19211-SP0772-L25261		**Exon 15**	
283	Reference probe 10285-L10797	2q		
291	**KANSL1** probe 19212-L25262		**Exon 9**	
301 Ж	**KANSL1** probe 19213-SP0773-L25263		**Exon 3**	
310	Reference probe 11316-L12042	12p		
319 « +	**KANSL1** probe 19214-L25264		upstream	
325	Reference probe 10711-L24066	6p		
331 Ж	**KANSL1** probe 19215-SP0774-L25734		**Exon 10**	
337 *	Reference probe 19093-L24980	4q		
345	**KANSL1** probe 19216-L25266		**Exon 4**	
351 ¬	MAPT probe 07857-L25765			centromeric
359 Ж	**KANSL1** probe 19217-SP0775-L26250		**Exon 13**	
364	Reference probe 16232-L24272	1p		

[a] See section Exon numbering on page 2 for more information.

* New in version A2.
« 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. 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.
+ This probe is located in the first exon of transcript variant 2 (NM_015443.4), 0.5 kb upstream of exon 1 of *KANSL1*.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. *KANSL1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*KANSL1* exon[a]	Ligation site NM_001193466.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*454-456 (Exon 2)*		
319 « +	19214-L25264	upstream	NM_015443.4; 552-553	GGCTGTTTGCAA-ACTGCGCCCATT	0.5 kb
133 «	S0991-L26452	Exon 1	348-349	CGAAACACACAT-CTTGGCGCGTTG	21.3 kb
238	19208-L25258	Exon 2	1498-1499	TGCTGACTCGAA-AGGCTGAAGCTG	0.2 kb
166	19201-L25921	Exon 2	1685-1686	AGGAGGGGAGTC-TGATATTGAAGA	76.2 kb
301 Ж	19213-SP0773-L25263	Exon 3	1763-1764; 1790-1791	GTCAGAATGGAA-27 nt spanning oligo-CAGCCGCTGGAA	0.1 kb
144	S0993-L26450	Exon 3	1884-intron 3	CGTGCTAATAAG-GTAAGGGATAAG	12.0 kb
345	19216-L25266	Exon 4	1902-1903	ATAGTTCTTGGG-GAGGTACCTCCC	0.1 kb
139	S0992-L26451	Exon 4	1984-1985	GGACTGATAAAT-TGGTAAGTATAA	14.7 kb
173	19202-L25252	Exon 5	44 nt before exon 5	CAGGCAGGGGTG-CGCTTGGGTACA	1.1 kb
154	19200-L25250	Exon 6	2235-2236	GCCCGGACACGT-CCTGTACTGAGC	16.0 kb
219	19207-L25257	Exon 7	2457-2456 *reverse*	GGAAATGCTAGA-ACAGGATGAACA	10.7 kb
188 Ж	19204-SP0770-L25254	Exon 8	2498-2499; 2528-2529	CCTGCATTTCCA-30 nt spanning oligo-GCCTTTTGACAA	0.7 kb
291	19212-L25262	Exon 9	2706-2707	AGGCAGCACTTA-GACGATGTGGGG	0.5 kb
331 Ж	19215-SP0774-L25734	Exon 10	2860-2861; 2893-2894	TGAAGCATCACA-33 nt spanning oligo-CCCACCATCCTC	4.5 kb
252	19209-L25766	Exon 11	2 nt after exon 11	TTACGCCCAGGT-AGAAGCTCAGAA	1.1 kb
	No probe	Exon 12			
359 Ж	19217-SP0775-L26250	Exon 13	3247-3248; 3280-3281	AGAGGGCACGGT-33 nt spanning oligo-GGGGCAGCAGGT	1.0 kb
181 Ж	19203-SP0769-L25733	Exon 14	3433-3434; 3458-3459	GGAGCCCCATTA-25 nt spanning oligo-CACCCCTGTGGC	0.7 kb
211	19206-L25256	Exon 15	3796-3797	ATCTAAACAGAC-TCACTAACTATT	0.3 kb
274 Ж	19211-SP0772-L25261	Exon 15	4022-4023; 4049-4050	TGAGACCCTGCT-27 nt spanning oligo-TGTTGAATCACT	12.5 kb
		stop codon	*3769-3771 (Exon 15)*		
351 ¬	07857-L25765		*MAPT* gene, exon 13	TCCAGTCGAAGA-TTGGGTCCCTGG	46.8 kb
202 ¬	08353-L08206		*MAPT* gene, exon 3	ATGTGTTCCAGA-ATCTCCCCTGCA	138.4 kb
160 ¬	08369-L14410		*CRHR1* gene, exon 11	CTCAGACGTGGT-GGATGCCCGGAG	49.0 kb
232 ¬	08367-L25922		*CRHR1* gene, exon 1	CGAGACGGAGCT-GCGGGTGCCCTC	

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.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. 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.
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SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.

- Steinberg KM et al. (2012). Structural diversity and African origin of the 17q21. 31 inversion polymorphism. *Nat Genet*. 44:872-880.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.
- Zollino M et al. (2015). Intragenic KANSL1 mutations and chromosome 17q21.31 deletions: broadening the clinical spectrum and genotype–phenotype correlations in a large cohort of patients. *J Med Genet*. 52:804-814.

Selected publications using SALSA MLPA Probemix P443 KANSL1

- León LE et al. (2017). Partial microduplication in the histone acetyltransferase complex member KANSL1 is associated with congenital heart defects in 22q11.2 microdeletion syndrome patients. *Sci Rep*. 7:1795.
- Marangi G et al. (2018). A novel truncating variant within exon 7 of KAT6B associated with features of both Say–Barber–Bieseker–Young–Simpson syndrome and genitopatellar syndrome: Further evidence of a continuum in the clinical spectrum of KAT6B-related disorders. *Am J Med Genet A*. 176:455-459.
- Miclea D et al. (2019). Genomic study via chromosomal microarray analysis in a group of Romanian patients with obesity and developmental disability/intellectual disability. *J Pediatr Endocrinol Metab*. 32:667-674.
- Sabo A et al. (2020). Community-based recruitment and exome sequencing indicates high diagnostic yield in adults with intellectual disability. *Mol Genet Genomic Med*. 8:e1439.
- Zollino M et al. (2015). Intragenic KANSL1 mutations and chromosome 17q21.31 deletions: broadening the clinical spectrum and genotype-phenotype correlations in a large cohort of patients. *J Med Genet*. 52:804-814.

P443 product history	
Version	*Modification*
A2	Three reference probes have been replaced and one reference probe has been removed.
A1	First release.

Implemented changes in the product description
Version A2-01– 16 December 2020 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *KANSL1* gene updated according to new versions of the NM_ reference sequences. *Version 02 – 18 August 2017 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - New references added on page 2. - Minor textual changes.

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