Product Description SALSA® MLPA® Probemix P324-B1 22q11

To be used with the MLPA General Protocol.

Version B1. As compared to version A2, twelve target probes have been replaced, and six have been removed. All reference probes have been replaced. Several probe lengths have been elongated, no change in sequence detected. For complete product history see page 7.

Catalogue numbers:
- **P324-025R:** SALSA MLPA Probemix P324 22q11, 25 reactions.
- **P324-050R:** SALSA MLPA Probemix P324 22q11, 50 reactions.
- **P324-100R:** SALSA MLPA Probemix P324 22q11, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

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

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P324 22q11 is a **research use only (RUO)** assay for the detection of deletions or duplications in the 22q11 chromosomal region. Microdeletions/-duplications in the 22q11.2 region cause a variety of disorders, including the 22q11.2 distal deletion syndrome (MIM 611867), the 22q11.2 microduplication syndrome (MIM 608363), DiGeorge syndrome (DGS; MIM 188400), velocardiofacial syndrome (VCFS; MIM 192430) and cat eye syndrome (CES; MIM 115470). We recommend the use of the SALSA MLPA Probemix P250 DiGeorge as a primary screening for DiGeorge syndrome.

The high frequency of 22q11.2 copy number changes is due to the presence of several low copy repeats (LCRs), labelled A-H, which facilitate misalignment. The overall birth prevalence of 22q11.2 deletions appears to be approximately 1 in 4,000. The 22q11.2 deletion syndrome is inherited in an autosomal dominant manner, with about 93% of probands having a *de novo* deletion, whereas the remaining 7% of probands have inherited the 22q11.2 deletion from a parent. The 22q11.2 deletion syndrome, which includes DGS and VCFS, most often (85%) results from a 3 Mb deletion on the 22q11.2 region. This 3 Mb deleted region is flanked by LCR-A and LCR-D, and includes the *TBX1* gene. The remaining 15% of affected individuals have smaller, atypical deletions, which can include LCR A-B, B-D or C-D deletions. Haploinsufficiency of the *TBX1* gene in particular is responsible for most of the physical malformations. Point mutations in this gene have also been observed in individuals with DGS. The phenotype of individuals with 22q11.2 deletion syndrome is characterised by palatal anomalies, congenital heart problems and distinct facial features. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1523/.

The 22q11.2 microduplication syndrome is inherited in an autosomal dominant manner. Most individuals with a 22q11.2 duplication have inherited the duplication from a parent, but *de novo* 22q11.2 duplications also occur. The penetrance of 22q11.2 duplication is incomplete. The phenotype is generally mild and highly variable. It is characterised by intellectual disability, delayed psychomotor development, growth retardation and/or hypotonia. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK3823/.

CES has a large clinical variability, ranging from marginally affected individuals to those with the full pattern of malformations and a lethal outcome. The eyes are predominantly affected. CES is caused by a small, dicentric, supernumerary chromosome 22 representing an inv dup (22)(q11). In many cases this chromosomal abnormality is mosaic.

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/

Probemix content: The SALSA MLPA Probemix P324-B1 22q11 contains 40 MLPA probes with amplification products between 130 and 463 nucleotides (nt). This includes 28 probes for the 22q11 chromosomal region, among which seven probes for the *TBX1* gene, and one probe for the 22q12.1 chromosomal region. In addition, 11 reference probes are included, detecting several different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

This Probemix contains nine quality control fragments generating amplification products between 64 and 121 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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)
88-96	D-fragments (Low signal of 88 or 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls result in only five major peaks shorter than 121 nt: four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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: 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 unrelated individuals who are from families without a history of 22q11.2 deletion syndrome or other conditions related to aberrations in the 22q11 chromosomal region. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

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 DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID numbers NA17942, NA07215 and NA10382 from the Coriell Institute have been tested at MRC-Holland and can be used as positive control samples to detect the typically deleted region (TDR) at 22q11.2, extending from LCR-A to LCR-D. Sample ID number NA05401 has been tested at MRC-Holland and can be used as a positive control sample to detect a

22q11.2 deletion (LCR-A – LCR-B) and deletion of the CES region. 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.mlpa.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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal regions:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. 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 (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 are unlikely to have any relation to the condition tested for.

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

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P324-B1 22q11

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	Chromosomal position (hg18) Target region	Location (hg18) in kb
64-105	Control fragments – see table in probemix content section for more information			
130 *	Reference probe 00797-L00463	5q31		05-132.038
136 «	**TBX1 probe** 09404-L09690		**22q11.21**	22-018.124
142 +	**BCRP2 (FLJ42953) probe** 06317-L10754		**22q11.21**	22-019.779
152 *	Reference probe 14199-L25033	2q13		02-108.894
160 *	**HIRA probe** 01214-L02328		**22q11.21**	22-017.699
170 ¥ «	**TBX1 probe** 22028-L30951		**22q11.21**	22-018.127
178 *	Reference probe 08599-L28166	17p11		17-017.059
184 *	**MICAL3 probe** 05458-L28169		**22q11.21**	22-016.705
196 «	**TBX1 probe** 09411-L10879		**22q11.21**	22-018.134
202 *	**CLDN5 probe** 01218-L28172		**22q11.21**	22-017.891
208 *	Reference probe 12490-L17096	1q32		01-208.032
214	**VPREB1 probe** 09403-L09689		**22q11.22**	22-020.930
228 *	**USP18 probe** 07528-L28175		**22q11.21**	22-017.013
238	**COMT probe** 07490-L10757		**22q11.21**	22-018.336
244 *	Reference probe 13389-L14846	6q12		06-064.489
251 *	**ZNF74 probe** 22035-L30956		**22q11.21**	22-019.085
260 ¥	**MIF probe** 22027-L30950		**22q11.23**	22-022.567
269 *	**BID probe** 22029-L16426		**22q11.21**	22-016.607
275	**SEZ6L probe** 05929-L05810		**22q12.1**	22-025.018
283 *	Reference probe 06754-L06358	8q12		08-061.912
292 «	**TBX1 probe** 09406-L09692		**22q11.21**	22-018.128
301 * «	**DGCR2 probe** 22036-L30957		**22q11.21**	22-017.406
307 *	**CDC45 probe** 22031-L05808		**22q11.21**	22-017.847
319	**GNB1L probe** 07487-L07145		**22q11.21**	22-018.156
328 *	Reference probe 02663-L02130	11q22		11-107.649
342 *	**IL17RA probe** 01082-L30960		**22q11.1**	22-015.960
349 ¥ «	**TBX1 probe** 09407-L31277		**22q11.21**	22-018.131
355 *	**BID probe** 22030-L16320		**22q11.21**	22-016.597
361 *	Reference probe 07034-L22804	14q11		14-020.889
369 «	**TBX1 probe** 09410-L10764		**22q11.21**	22-018.133
374	**COMT probe** 07489-L10761		**22q11.21**	22-018.330
382	**DGCR8 probe** 08476-L10765		**22q11.21**	22-018.478
391 *	**CECR2 probe** 13953-L15492		**22q11.21**	22-016.337
400 *	Reference probe 07678-L06854	7p15		07-030.970
409 ¥	**TBX1 probe** 22032-L09699		**22q11.21**	22-018.151
418	**PRODH probe** 07486-L07144		**22q11.21**	22-017.299
436 *	Reference probe 09614-L09909	20p12		20-010.573
445 ¥	**RIMBP3C probe** 22033-L05796		**22q11.21**	22-020.247
453 *	**SLC25A18 probe** 06220-L04860		**22q11.21**	22-016.423
463 *	Reference probe 00979-L21331	10p14		10-012.019

* New in version B1 (from lot B1-1118 onwards).

¥ Changed in version B1 (from lot B1-1118 onwards). Small change in length, no change in sequence detected.

« 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.

+ The name of this gene has changed. Previous name can be found between brackets.

Table 2. 22q11 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
Cat eye syndrome (CES) region:				
342 »	01082-L30960	IL17RA	GCAGAGTTATCT-GTCCTGCAGCTG	377.0 kb
391	13953-L15492	CECR2	TGACGTGGAGTT-TATCAGTGACCT	86.7 kb
453 »	06220-L04860	SLC25A18	GCAGTGAGAAGA-GTCGAGTGAAGC	173.9 kb
355	22030-L16320	BID	GTCCCAGGAATC-AGTTTAAAAGCA	9.5 kb
269 »	22029-L16426	BID	CTACTGGTGTTT-GGCTTCCTCCAA	98.0 kb
184 »	05458-L28169	MICAL3	GAACTACCGCCT-GTCCCTGAGGCA	308.3 kb
228 »	07528-L28175	USP18	CTCAGTCCCGAC-GTGGAACTCAGC	285.6 kb
End of CES region; Start DiGeorge region; probes in region LCR22A – LCR22B:				
418	07486-L07144	PRODH	ACTACAGGGCCT-TCGGTGTCAGCG	107.6 kb
301 «	22036-L30957	DGCR2	CATAGCTTTGGA-TCACTGTCTTCT	292.9 kb
160 »	01214-L02328	HIRA	GGAGCTGCTGAA-GGAGCTGCTACC	148.5 kb
307 »	22031-L05808	CDC45	ATGTTCGTGTCC-GATTTCCGCAAA	43.8 kb
202 »	01218-L28172	CLDN5	TTCGCCAACATT-GTCGTCCGCGAG	232.9 kb
136 «	09404-L09690	TBX1	AGGGAGGGAGGA-ACACTTGCCGCG	2.8 kb
170 «, »	22028-L30951	TBX1	CCGGGTGAAGCT-TCGCTGGCTGCC	1.3 kb
292 «	09406-L09692	TBX1	AGCTCTCGCATT-TCTGCGACGTTG	2.4 kb
349 «	09407-L31277	TBX1	TCCCACCTTCCA-AGTGAAGCTCTT	2.5 kb
369 «, »	09410-L10764	TBX1	TCCCTTCGCGAA-AGGCTTCCGGGA	0.2 kb
196 «	09411-L10879	TBX1	CGGCACGGAGAA-AGGTAGGGCCGG	17.3 kb
409	22032-L09699	TBX1	AAGTCAGGAGGT-CAAGTGTGCATG	5.6 kb
319	07487-L07145	GNB1L	CGGGATCGCCGA-GGTCACGATCCG	173.9 kb
374	07489-L10761	COMT	TTGACACCTACT-GCGAGCAGAAGG	5.8 kb
238	07490-L10757	COMT	GTGCGCCAGACT-TCCTAGCACACG	141.7 kb
382	08476-L10765	DGCR8	GACTCAGCGACT-GCACCAGTGGCA	607.0 kb
Probes in region LCR22B – LCR22D:				
251	22035-L30956	ZNF74	TGAGAACCTATA-TGTCATTTCAGG	694.6 kb
142	06317-L10754	BCRP2 (FLJ42953)	TGAAATCTTGTA-GAGTACAGACAG	467.7 kb
End of the commonly-deleted DiGeorge region; probes in region LCR22D – LCR22E:				
445	22033-L05796	RIMBP3C	CTGGGCCCAAGG-CCTAATAGGTGA	682.7 kb
214	09403-L09689	VPREB1	CTGAGCCACTCA-GCATCTCCTGGT	1.6 Mb
Probes in region LCR22G and downstream:				
260	22027-L30950	MIF	CATGCCGATGTT-CATCGTAAACAC	2.5 Mb
275	05929-L05810	SEZ6L	ACAGTCGGCGGA-AGTGCTGGGCGA	

« 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.
» Detects the same sequence as a probe in SALSA MLPA Probemix P250 DiGeorge.

Related SALSA MLPA probemixes

- P064 Microdeletion Syndromes-1B: Contains probes for the 22q11 DiGeorge region.
- P245 Microdeletion Syndromes-1A: Contains probes for the 22q11 DiGeorge region.
- P250 DiGeorge: Primary screening of the DiGeorge region.
- P258 SMARCB1: Contains probes for the *SMARCB1* gene at 22q11.23, associated with rhabdoid tumours.
- P372 Microdeletion Syndromes 6: Contains probes for the 22q11 DiGeorge region.
- P455 LZTR1: Contains probes for the *LZTR1* gene at 22q11.21, associated with schwannomatosis.
- P463 MRKH: Contains probes for the *TBX1* gene at 22q11.21, associated with Mayer-Rokitansky-Küster-Hauser syndrome.

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.
- 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.

Selected publications using SALSA MLPA Probemix P324 22q11

- Evers LJ et al. (2016). The use of two different MLPA kits in 22q11.2 Deletion Syndrome. *Eur J Med Genet.* 59: 183-188.
- Fernández L et al. (2009). A deletion and a duplication in distal 22q11.2 deletion syndrome region. Clinical implications and review. *BMC Med Genet.* 10:48.

P324 Product history	
Version	*Modification*
B1	Twelve target probes have been replaced, and six have been removed. All reference probes have been replaced. Several probe lengths have been elongated, no change in sequence detected.
A2	The 88 and 96 nt control fragments have been replaced. Two control fragments at 100-105 nt have been included.
A1	First release.

Implemented changes in the product description

Version B1-01 – 07 January 2019 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- For uniformity, the chromosomal positions and bands in this document are now all based on hg18 (NCBI36).
- Related SALSA MLPA probemixes section was updated.
- Probemix name changed to 22q11 (was 22q11 mix-2).

Version 10 – 28 September 2017 (55)
- Warning added in Table 1, 391 nt probe 05459-L04862.

Version 09 (55) – 03 December 2015
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new pictures included).
- Manufacturer's address adjusted.
- "Peak area" replaced with "peak height".
- Various minor textual changes.

Version 08 (48)
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.

Version 07 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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