Product Description SALSA® MLPA® Probemix P369-A2 Smith-Magenis Syndrome

To be used with the MLPA General Protocol.

Version A2. For complete product history see page 6.

Catalogue numbers:
- **P369-025R:** SALSA MLPA Probemix P369 Smith-Magenis, 25 reactions.
- **P369-050R:** SALSA MLPA Probemix P369 Smith-Magenis, 50 reactions.
- **P369-100R:** SALSA MLPA Probemix P369 Smith-Magenis, 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 P369 Smith-Magenis syndrome is a **research use only (RUO)** assay for the detection of deletions or duplications in the 17p11.2 chromosomal region, including *RAI1*. Deletion of this region is associated with Smith-Magenis syndrome (SMS), whereas duplication of this region is associated with Potocki-Lupski syndrome (PTLS). Furthermore, two probes targeting the 2q37.3 region are included, among which one probe for the *HDAC4* gene. Haploinsufficiency (either by a mutation or deletion) of the *HDAC4* gene causes Brachydactyly-mental retardation syndrome, which has an overlapping phenotype with SMS. Furthermore, deletion or mutation of the *HDAC4* gene results in reduced expression of *RAI1* (Williams et al. 2010).

SMS is a developmental disorder characterised by craniofacial anomalies, and several neurological and behavioural abnormalities. It is primarily caused by an interstitial deletion on chromosome 17p11.2 which comprises multiple genes, including *RAI1*. In most cases (~70%), SMS is due to a ~3.7 Mb deletion, but atypical (smaller or larger) deletions, as well as *RAI1* mutations have also been found in patients. Haploinsufficiency of *RAI1* is therefore thought to play a major role in SMS (Elsea & Girirajan 2008).

Duplication of the same ~3.7 Mb region is associated with PTLS, which shows some phenotypical overlap with SMS, yet the clinical features of each syndrome are somewhat distinct. As in SMS, size of the duplication can vary between patients. The prevalence for SMS and PTLS is approximately 1 in 25,000 (Neira-Fresneda & Potocki 2015).

More information is available at: https://www.ncbi.nlm.nih.gov/books/NBK1310/ (SMS) & https://www.ncbi.nlm.nih.gov/books/NBK447920/ (PTLS).

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 P369-A2 Smith-Magenis syndrome contains 35 MLPA probes with amplification products between 133 and 429 nt. This includes 23 probes for the 17p11.2 chromosomal region, of which eight target the *RAI1* gene. Furthermore, two probes for the 2q37.3 chromosomal region are also present. In addition, ten reference probes are included and detect ten 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 results in only five major peaks shorter than 121 nucleotides (nt): 4 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 4 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 SMS or PTLS. 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 Biobank (https://catalog.coriell.org) has a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number NA13476 from the Coriell Institute has been tested at MRC-Holland and can be used as a positive control sample to detect the common chromosome 17p11.2 deletion indicated in Table 2a. 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 chromosomes:

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 (e.g., a duplication of *RAI1* exons 3 and 5 but not exon 4) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P369-A2 Smith-Magenis Syndrome

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		
		Reference	SMS region	2q37
64-105	Control fragments – see table in probemix content section for more information			
133	Reference probe 17941-L22850	1q32		
139 ¬	**ZNF624 probe** 01447-L00931		Telomeric	
146	**RAI1 probe** 16525-L15418		**RAI1** exon 2	
160	**RAI1 probe** 16526-L19755		**RAI1** exon 6	
166	**TNFRSF13B probe** 01448-L00932		**TNFRSF13B**	
172	**RAI1 probe** 16586-L19088		**RAI1** exon 5	
178	Reference probe 08731-L08742	9q21		
184	**TOM1L2 probe** 04669-L04051		**TOM1L2**	
202	**FLCN probe** 08587-L08588		**FLCN**	
209 «	**MIR33B probe** 16527-L19756		**MIR33B**	
215	Reference probe 06539-L06097	5q13		
221	**NDUFA10 probe** 09034-L09288			**NDUFA10**
227 ¬	**USP22 probe** 15143-L21447		Centromeric	
234	**AKAP10 probe** 16528-L21448		**AKAP10**	
241 «	**RAI1 probe** 16587-L19089		**RAI1** exon 1	
250	**RAI1 probe** 16588-L21735		**RAI1** exon 2	
256	**SPECC1 probe** 16529-L21736		**SPECC1**	
263	Reference probe 07391-L21737	12q13		
268	**FLCN probe** 08600-L21738		**FLCN**	
275	**DRC3 (LRRC48) probe** 01452-L21174		**DRC3 (LRRC48)**	
283	**COPS3 probe** 09361-L19757		**COPS3**	
292	Reference probe 17265-L20654	6q23		
302	Reference probe 05285-L04666	14q22		
312 «	**LLGL1 probe** 01453-L19758		**LLGL1**	
322	Reference probe 15962-L18114	9q34		
332	**PRPSAP2 probe** 01454-L14433		**PRPSAP2**	
346	Reference probe 09108-L09167	4q25		
364	**MFAP4 probe** 01455-L14554		**MFAP4**	
378	**ALDH3A1 probe** 06243-L05749		**ALDH3A1**	
385	Reference probe 05914-L05359	18p11		
393	**HDAC4 probe** 10036-L11449			**HDAC4**
402 Ж	**RAI1 probe** 16589-SP0377-L21449		**RAI1** exon 4	
412 «	**RAI1 probe** 16590-L21450		**RAI1** exon 1	
421	**RAI1 probe** 16591-L21009		**RAI1** exon 3	
429	Reference probe 15541-L21452	2q22		

« 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.

¬ 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.

Note: The exon numbering used in this P369-A2 Smith-Magenis syndrome product description is the exon numbering from the RefSeq transcript NG_007101.2 (NM_030665.3). The exon numbering and NM sequence used is from 09/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Note: The gene name of *LRRC48* has changed to *DRC3* from description version A2-01 onwards The gene name used in previous versions of the product description can be found between brackets in Table 1 and Table 2. Please notify us of any mistakes: info@mlpa.com.

Table 2. P369-A2 probes arranged according to chromosomal location

Table 2a. 17p11.2

Length (nt)	SALSA MLPA probe	Gene/ Exon	Ligation site RAI1	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
139 ¬	01447-L00931	ZNF624		TACACCAGGCTG-TTAATATTGATT	327.9 kb
Start of common chromosome 17p11.2 deletion/duplication region					
166	01448-L00932	TNFRSF13B		GAGCAAGGCAAG-TTCTATGACCAT	266.1 kb
268	08600-L21738	FLCN		CGTCTGCCTCAA-GGAGGAGTGGAT	22.1 kb
202	08587-L08588	FLCN		GTCGCTCCTGGT-TCTGCCAGCTCC	10.4 kb
283	09361-L19757	COPS3		GCTGAAAGCCAT-GGACCAGGAGAT	434.2 kb
		RAI1	**NM_030665.3**		
		start codon	*470-472 (exon 3)*		
412 «	16590-L21450	Exon 1	281-282	CGCGAAGTCGCA-GCGCCAGACCCA	0.1 kb
241 «	16587-L19089	Exon 1	98 nt after exon 1	GTGATGAGCCGA-GGCGGGTTCGGA	42.1 kb
146	16525-L15418	Exon 2	328-329	CCAAGGATCTCA-TCTGGCCACCGC	0.3 kb
250	16588-L21735	Exon 2	204 nt after exon 2	GTTTTCCACGCT-GGTTGAGGGAAT	68.6 kb
421	16591-L21009	Exon 3	487-488	TCTTTTCGAGAA-AGGTGTGGTTTC	10.7 kb
402 Ж	16589-SP0377-L21449	Exon 4	45 nt and 18 nt before exon 4	AGCTTGAGGGCT- 27 nt spanning oligo -TTCCTTTCTCTT	5.7 kb
172	16586-L19088	Exon 5	6175-6176	TGTCCCAAACAT-AAGGTAGGGGAC	1.5 kb
160	16526-L19755	Exon 6	7092-7093	CAGCGCTAGATT-TCGTGTACAAAA	3.2 kb
		stop codon	*6188-6190 (exon 6)*		
209 «	16527-L19756	MIR33B		AGACCCTGCTTT-TTGGCTAAGGCT	158.4 kb
184	04669-L04051	TOM1L2		GACAGAGGTGTA-ACGACCAATAGG	15.5 kb
275	01452-L21174	DRC3 (LRRC48)		CGGATCTCCAAG-ATCGACTCCCTG	244.7 kb
312 «	01453-L19758	LLGL1		CAGCAGTCTGCA-TCTCTGGGAGAT	633.2 kb
332	01454-L14433	PRPSAP2		TAGAAACCAAGA-TGAACATAACCA	518.6 kb
364	01455-L14554	MFAP4		TGCCAACCTCAA-TGGCTTCTACCT	353.8 kb
378	06243-L05749	ALDH3A1		AGCTTCGAGACT-TTCTCTCACCGC	224.5 kb
234	16528-L21448	AKAP10		AGGACCAAGTCA-TGTTGCAATCAA	264.6 kb
256 #	16529-L21736	SPECC1		AACCATATTTGA-ATTGGAAGATCA	801.0 kb
End of common chromosome 17p11.2 deletion/duplication region					
227 ¬	15143-L21447	USP22		GGCTGTTTCACA-AAGAAGCATATT	

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

« 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.

¬ 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.

Table 2b. 2q37

Length (nt)	SALSA MLPA probe	Gene	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
393	10036-L11449	HDAC4		GAGTTTGGAGCT-CGTTGGAGCTAT	669.9 kb
221	09034-L09288	NDUFA10		CCTTGGAGCACT-TGCTGACCACAG	

Note: The exon numbering used in this P369-A2 Smith-Magenis syndrome product description is the exon numbering from the RefSeq transcript NG_007101.2 (NM_030665.3). The exon numbering and NM sequence used is from 09/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Note: The gene name of *LRRC48* has changed to *DRC3* from description version A2-01 onwards The gene name used in previous versions of the product description can be found between brackets in Table 1 and Table 2. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P029 Williams-Beuren syndrome.
- P064 Microdeletion Syndromes-1B: Probes for 15 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
- P245 Microdeletion Syndromes-1A: Probes for 23 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
- P250 DiGeorge syndrome.
- P371-372-373-374: Probemixes that can be used to confirm P245 results.
- ME028 Prader-Willi/Angelman syndrome.

References

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- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
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P369 Product history	
Version	*Modification*
A2	One reference probe has been added and two have been replaced.
A1	First release

Implemented changes in the product description
Version A2-01 – 26 September 2018 (01P) - Product description restructured and adapted to a new template. - Various minor textual or layout changes. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - *LRRC48* gene name updated to *DRC3* in Table 1 and Table 2. *Version 04 – 21 August 2015 (55)* - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new pictures included). *Version 03 (53)* - Warning added in Table 1, 312 nt probe 01453-L19758 added.

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