Product Description

SALSA® MLPA® Probemix P369-B1 Smith-Magenis

To be used with the MLPA General Protocol.

Version B1

As compared to version A2, one target probe has been replaced, ten target probes have been added, five reference probes have been replaced, two reference probes have been added and one reference and three target probes have been changed in length, not in sequence detected. For complete product history see page 8.

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

SALSA® MLPA® Probemix P369 Smith-Magenis (hereafter: P369 Smith-Magenis) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P369-025R	**P369-050R**	**P369-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix 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).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, 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.

Certificate of Analysis

Information regarding 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

SALSA® MLPA® Probemix P369 Smith-Magenis is a **research use only (RUO)** assay for detection of deletions or duplications in the 17p11.2 chromosomal region. Deletion of this region is associated with Smith-Magenis

syndrome (SMS), whereas duplication of this region is associated with Potocki-Lupski syndrome (PTLS). 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 where the gene *RAI1*, included in this probemix, is located. 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 (Elsea & Girirajan 2008).

Haploinsufficiency of *RAI1* is therefore thought to play a major role in the SMS phenotype (Elsea & Girirajan 2008). Other genes located in the 17p11.2 critical region that have also been implicated to play a role in some SMS features, namely *MY015A* (deafness), *TNFRSF13B* (immune deficiency), *PEMT* (fatty liver), and *ALDH3A2* (dry skin), as well as *PMP22* (neuropathy development) which is flanking this region, are also covered (Vilboux et al. 2011 and Poisson et al. 2019).

Furthermore, five probes targeting the 2q37.3 region are included, among which probes targeting the *HDAC4* gene. Haploinsufficiency (either by a mutation or deletion) of the *HDAC4* gene causes chromosome 2q37 deletion 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, Trang et al. 2019).

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) and https://www.ncbi.nlm.nih.gov/books/NBK447920/ (PTLS).

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE
Tark – Transcript Archive: https://tark.ensembl.org

Exon numbering
The *RAI1* exon numbering used in this P369-B1 Smith-Magenis product description is the exon numbering derived from MANE project (release version 1.0) based on MANE Select transcript NM_030665.4.

The *HDAC4* exon numbering used in this P369-B1 Smith-Magenis product description is the exon numbering derived from MANE project (release version 1.0) based on MANE Select transcript NM_001378414.1.

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
P369-B1 Smith-Magenis contains 47 MLPA probes with amplification products between 127 and 483 nucleotides (nt). This includes one probe for the 17p12 chromosomal region and 29 probes for the 17p11.2 chromosomal region, of which ten target the *RAI1* gene. Furthermore, five probes for the 2q37.3 chromosomal region are also present. In addition, twelve reference probes are included and detect twelve different 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 MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from 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 (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. Sample ID number NA13476 from the Coriell Institute has been tested with P369-B1 Smith-Magenis 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 deviations to the indicated copy number alteration (CNA) findings might occur.

Sample name	Source	Chromosomal position (hg18) of copy number alteration*	Altered target genes in P369-B1 Smith-Magenis	Expected copy number alteration

NA13476	Coriell Institute	17p11	*TNFRSF13B, FLCN, COPS3, PEMT, RAI1, MIR33B, TOM1L2, DRC3, MYO15A, LLGL1, PRPSAP2, MFAP4, ALDH3A2, ALDH3A1, AKAP10, , SPECC1..*	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by P369-B1 Smith Magenis.

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 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/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also 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 (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.

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 in sequence data 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.

***RAI1and HDAC4* mutation database**
https://databases.lovd.nl/shared/genes/RAI1 and https://databases.lovd.nl/shared/genes/HDAC4. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://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 *RAI1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. P369-B1 Smith-Magenis

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]		
		Reference	SMS region	2q37
64-105	Control fragments – see table in probemix content section for more information			
127 *	Reference probe 20074-L20313	11p		
132 *	**TNFRSF13B probe** 22860-L32248		***TNFRSF13B***	
139 ¬	**ZNF624 probe** 01447-L00931		Telomeric	
146	**RAI1 probe** 16525-L15418		***RAI1*** exon 2	
153 *	Reference probe 18804-L24300	12q		
160	**RAI1 probe** 16526-L19755		***RAI1*** exon 6	

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]		
		Reference	SMS region	2q37
166	**TNFRSF13B probe** 01448-L00932		***TNFRSF13B***	
173 ¥	**RAI1 probe** 16586-L33034		***RAI1*** exon 5	
179 ¥	Reference probe 08731-L33000	09q		
184	**TOM1L2 probe** 04669-L04051		***TOM1L2***	
190 *	**ALDH3A2 probe** 22861-L32249		***ALDH3A2***	
197 * ¬	**PMP22 probe** 22862-L32250		Telomeric	
202	**FLCN probe** 08587-L08588		***FLCN***	
209	**MIR33B probe** 16527-L19756		***MIR33B***	
215 *	Reference probe 22513-L31662	14q		
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*** intron 2	
256 #	**SPECC1 probe** 16529-L21736		***SPECC1***	
262 *	Reference probe 18212-L33076	08p		
268	**FLCN probe** 08600-L21738		***FLCN***	
275	**DRC3 probe** 01452-L21174		***DRC3***	
283	**COPS3 probe** 09361-L19757		***COPS3***	
292	Reference probe 17265-L20654	06q		
298 *	**PEMT probe** 22863-L32251		***PEMT***	
312 «	**LLGL1 probe** 01453-L19758		***LLGL1***	
322	Reference probe 15962-L18114	09q		
332	**PRPSAP2 probe** 01454-L14433		***PRPSAP2***	
343 *	Reference probe 13869-L30647	07p		
355 *	**RAI1 probe** 22866-L32254		***RAI1*** exon 4	
364	**MFAP4 probe** 01455-L14554		***MFAP4***	
371 *	**HDAC4 probe** 22867-L32255			***HDAC4*** exon 12
379 ¥	**ALDH3A1 probe** 06243-L33001		***ALDH3A1***	
385	Reference probe 05914-L05359	18p		
395 ¥	**HDAC4 probe** 10036-L30116			***HDAC4*** exon 2
403 *	**MYO15A probe** 22868-L32256		***MYO15A***	
412	**RAI1 probe** 16590-L21450		***RAI1*** exon 1	
421	**RAI1 probe** 16591-L21009		***RAI1*** exon 3	
429	Reference probe 15541-L21452	02q		
436 *	**RAI1 probe** 22869-L32257		***RAI1*** exon 3	
445 *	Reference probe 20431-L27913	01q		
452 *	**HDAC4 probe** 22870-L32258			***HDAC4*** exon 7
463 *	**RAI1 probe** 11733-L13832		***RAI1*** exon 3	
475 *	**HDAC4 probe** 22871-L32259			***HDAC4*** exon 26
483 *	Reference probe 19180-L01344	13q		

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

* New in version B1
¥ Changed in version B1. Minor alteration, 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.
¬ 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's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

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

Table 2. Target and flanking probes arranged according to chromosomal location

Table 2a. 17p12 and 17p11.2

Length (nt)	MLPA probe	Gene / exon[a]	Ligation site *RAI1*[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
			17p12		
197 ¬	22862-L32250	*PMP22*		CTCAACTCGGAT-TACTCCTACGGT	1.4 **M**b
			17p11.2		
139 ¬	01447-L00931	*ZNF624*		TACACCAGGCTG-TTAATATTGATT	318.6 kb
Start of common chromosome 17p11.2 deletion/duplication region					
132	22860-L32248	*TNFRSF13B*		CACAGTCCTGCA-GCCTTGCCCACA	9.3 kb
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	258.3 kb
298	22863-L32251	*PEMT*		GGGTCCCACAAG-AGGAGCTGATTG	176.0 kb
		RAI1	**NM_030665.4**		
		start codon	*485-487 (exon 3)*		
412	16590-L21450	Exon 1	296-297	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	343-344	CCAAGGATCTCA-TCTGGCCACCGC	0.3 kb
250	16588-L21735	Intron 2	204 nt after exon 2	GTTTTCCACGCT-GGTTGAGGGAAT	68.6 kb
421	16591-L21009	Exon 3	502-503	TCTTTTCGAGAA-AGGTGTGGTTTC	1.7 kb
463	11733-L13832	Exon 3	2198-2199	CTGACGACTCCT-TCCAGAGCCTAC	3.4 kb
436	22869-L32257	Exon 3	5590-5591	CTTGGGGACCTC-TGTGGGCCCTAC	5.8 kb
355	22866-L32254	Exon 4	2 nt after exon 4	AGCGATGCAGGT-ACGAGCCCGCCC	5.6 kb
173	16586-L33034	Exon 5	6190-6191	TGTCCCAAACAT-AAGGTAGGGGAC	1.5 kb
160	16526-L19755	Exon 6	7107-7108	CAGCGCTAGATT-TCGTGTACAAAA	3.2 kb
		stop codon	*6203-6205 (exon 6)*		
209	16527-L19756	*MIR33B*		AGACCCTGCTTT-TTGGCTAAGGCT	158.4 kb
184	04669-L04051	*TOM1L2*		GACAGAGGTGTA-ACGACCAATAGG	15.5 kb
275	01452-L21174	*DRC3*		CGGATCTCCAAG-ATCGACTCCCTG	170.6 kb
403	22868-L32256	*MYO15A*		TTGCCCAGAAGT-ATTTCCGAGACC	74.1 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	267.1 kb
190	22861-L32249	*ALDH3A2*		GGGTTACTGCTA-AACCAGTTAAGA	86.8 kb
379	06243-L33001	*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	

Table 2b. 2q37

Length (nt)	MLPA probe	Gene / exon[a]	Ligation site *HDAC4*[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
221	09034-L09288	*NDUFA10*		CCTTGGAGCACT-TGCTGACCACAG	669.9 kb
		HDAC4	**NM_001378414.1**		
		start codon	*263-265 (exon 2)*		
395	10036-L30116	Exon 2	191-192	GAGTTTGGAGCT-CGTTGGAGCTAT	196.0 kb

Length (nt)	MLPA probe	Gene / exon[a]	Ligation site *HDAC4*[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
452	22870-L32258	Exon 7	887-888	AAACGCAGCACA-GTTCCCTTGACC	30.1 kb
371	22867-L32255	Exon 12	1574-1575	ATCTTTCAGGCC-TGGGAGCACTGC	73.1 kb
475	22871-L32259	Exon 26	3412-3413	CTGATCGAGGCT-CAGACTTGCGAG	
		stop codon	*3530-3532 (exon 27)*		

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] 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.
¬ 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's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. 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, pseudogene or highly similar region.

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

Related products
For related products, see the product page on our website.

References

- Elsea SH & Girirajan S (2008). Smith-Magenis syndrome. *Eur J Hum Genet*. 6(4):412-21.
- Neira-Fresneda J & Potocki L (2015). Neurodevelopmental Disorders Associated with Abnormal Gene Dosage: Smith–Magenis and Potocki–Lupski Syndromes. *J Pediatr Genet.* 4(3): 159–167.
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- Williams S et al. (2010). Haploinsufficiency of *HDAC4* Causes Brachydactyly Mental Retardation Syndrome, with Brachydactyly Type E, Developmental Delays, and Behavioral Problems. *Am J Hum Genet.* 87(2): 219–228.

Selected publications using P369 Smith-Magenis

- Sironi et al. (2022). A unique Smith-Magenis patient with a de novo intragenic deletion on the maternally inherited overexpressed RAI1 allele. *Eur J Hum Genet.* 30(11): 1233–1238

P369 product history	
Version	*Modification*
B1	One target probe has been replaced, ten target probes have been added, five reference probes have been replaced, two reference probes have been added and one reference and three target probes have been changed in length, not in sequence detected.
A2	One reference probe has been added and two have been replaced.
A1	First release.

Implemented changes in the product description
Version B1-01 – 14 June 2024 (05P) - 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 *RAI1* gene updated according to new version of the NM_ reference sequence. - Selected publication added.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200