Product Description
SALSA® MLPA® Probemix P355-B1 Microcephaly

To be used with the MLPA General Protocol.

Version B1
As compared to version A2, one probe targeting the MCPH1 gene has been removed from the probemix. For complete product history see page 8.

Catalogue numbers:
- **P355-025R:** SALSA MLPA Probemix P355 Microcephaly, 25 reactions.
- **P355-050R:** SALSA MLPA Probemix P355 Microcephaly, 50 reactions.
- **P355-100R:** SALSA MLPA Probemix P355 Microcephaly, 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 P355 Microcephaly is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MCPH1*, *MCPH2* (*WDR62*), *MCPH3* (*CDK5RAP2*), *MCPH5* (*ASPM*), *MCPH6* (*CENPJ*), and *MCPH7* (*STIL*) genes, which are associated with primary microcephaly.

Primary microcephaly is characterised by a smaller than normal brain size and variable degree of intellectual disability (Mahmood et al. 2011). It is a genetically heterogeneous disorder, inherited in an autosomal recessive fashion. The incidence is ~1 in 10,000 in consanguineous populations and less in non-consanguineous populations. Defects in the abovementioned genes can be the cause of primary microcephaly. The proteins encoded by these genes all associate with the centrosome, for at least part of the cell cycle (Thornton et al. 2009).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK9587/

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 *MCPH1*, *WDR62*, *CDK5RAP2*, *ASPM*, *CENPJ*, and *STIL* exon numbering used in this P355-B1 Microcephaly product description is the exon numbering from the NG_016619.2, NG_028101.1, NG_008999.1, NG_015867.1, NG_009165.2, and NG_012126.1 sequences, respectively. 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 P355-B1 Microcephaly contains 46 MLPA probes with amplification products between 130 and 499 nucleotides (nt). This includes eight probes for the *MCPH1* gene, one probe for the *WDR62* gene, six probes for the *CDK5RAP2* gene, 14 probes for the *ASPM* gene, four probes for the *CENPJ* gene, and four probes for the *STIL* 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 probes over the experiment.

Required specimens
Extracted DNA, which includes DNA derived from paraffin-embedded tissues, 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 primary microcephaly. 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 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. 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

- In most populations, the major cause of genetic defects in the aforementioned genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P355 Microcephaly.
- 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.

LOVD mutation database

https://databases.lovd.nl/shared/genes. 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 http://varnomen.hgvs.org/.

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

Table 1. SALSA MLPA Probemix P355-B1 Microcephaly

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*MCPH1*	*CDK5RAP2*	*ASPM*	Other
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 00797-L19287	5q				
136	**MCPH1 probe** 15096-L16867		**Exon 14**			
142	**CDK5RAP2 probe** 15097-L16868			**Exon 31**		
148	**ASPM probe** 15098-L16869				**Exon 20**	
154	**WDR62 probe** 16812-L19467					***WDR62* Exon 1**
159	**CDK5RAP2 probe** 15099-L18787			**Exon 11**		
166	**MCPH1 probe** 15100-L16871		**Exon 2**			
171	**ASPM probe** 15101-L18695				**Exon 19**	
177 «	**CDK5RAP2 probe** 16144-L16873			**Exon 14**		
187	**MCPH1 probe** 20613-L16874		**Exon 10**			
195	**CDK5RAP2 probe** 15104-L16875			**Exon 35**		
202	**ASPM probe** 15105-L16876				**Exon 6**	
208	**CENPJ probe** 15106-L16877					***CENPJ* Exon 4**
214	Reference probe 08940-L09035	11p				
220	**ASPM probe** 15107-L16878				**Exon 24**	
226	**STIL probe** 12543-L13593					***STIL* Exon 3**
232	**MCPH1 probe** 15108-L16879		**Exon 4**			
238	**ASPM probe** 15109-L16880				**Exon 10**	
244	**CDK5RAP2 probe** 15110-L16881			**Exon 22**		
250	Reference probe 09702-L13325	17q				
265	**ASPM probe** 15111-L16882				**Exon 23**	
274	**STIL probe** 15112-L16883					***STIL* Exon 6**
292	**ASPM probe** 15114-L16885				**Exon 18**	
301	Reference probe 08014-L09988	7q				
310	**CENPJ probe** 15115-L16886					***CENPJ* Exon 2**
319	**ASPM probe** 15116-L16887				**Exon 14**	
328	**CDK5RAP2 probe** 15117-L16888			**Exon 3**		
337	**MCPH1 probe** 07150-L06762		**Exon 13**			
346	**ASPM probe** 15118-L16889				**Exon 28**	
355	Reference probe 12574-L13624	20p				
364	**STIL probe** 12557-L13607					***STIL* Exon 14**
373	**ASPM probe** 15119-L16890				**Exon 3**	
382	**MCPH1 probe** 15120-L16891		**Exon 11**			
391	Reference probe 07808-L22560	3p				
402	**ASPM probe** 15121-L16892				**Exon 13**	
407	Reference probe 14840-L16548	10q				
416	**CENPJ probe** 15122-L16893					***CENPJ* Exon 7**
427	**ASPM probe** 15123-L16894				**Exon 7**	
436	**MCPH1 probe** 15124-L16895		**Exon 13**			
445	**STIL probe** 15125-L19244					***STIL* Exon 12**
454	Reference probe 14844-L16552	18q				
463	**ASPM probe** 15126-L16897				**Exon 11**	
472	**CENPJ probe** 15127-L16898					***CENPJ* Exon 13**
484	**MCPH1 probe** 15128-L16899		**Exon 9**			
492	**ASPM probe** 15129-L16900				**Exon 8**	
499	Reference probe 09870-L15194	2p				

[a] See section Exon numbering on page 1 for more information.

« 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.

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. P355-B1 probes arranged according to chromosomal location

Table 2a. *STIL*

Length (nt)	SALSA MLPA probe	*STIL* exon[a]	Ligation site NM_001048166.1	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*156-158 (Exon 2)*		
226	12543-L13593	Exon 3	231-232	TACCTTTCCACT-TTCCTCCATCAA	4.9 kb
274	15112-L16883	Exon 6	668-669	CTGCTTTCCCTA-AGAGTTCATATC	19.1 kb
445	15125-L19244	Exon 12	1660-1661	TCTTTTGAGACA-CTGCAAAGTAAG	11.1 kb
364	12557-L13607	Exon 14	2564-2565	TGGAATGCAGCA-GGTGAGGATCAA	
		stop codon	*4020-4022 (Exon 17)*		

Table 2b. *ASPM*

Length (nt)	SALSA MLPA probe	*ASPM* exon[a]	Ligation site NM_018136.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*233-235 (Exon 1)*		
373	15119-L16890	Exon 3	921-922	TGCTTTCAATGA-ATGCCATGGTGC	10.2 kb
202	15105-L16876	Exon 6	2623-2624	TTAATTGTTCGA-AAAGATAGACAC	1.0 kb
427	15123-L16894	Exon 7	2665-2666	GAACGTCAGAAA-GTCCTGAATTGG	2.3 kb
492	15129-L16900	Exon 8	2760-2761	AGATAACAGTGA-TGTCACAGGGTT	1.5 kb
238	15109-L16880	Exon 10	3108-3109	GACACCATTTGA-TGAATTTGATTT	3.4 kb
463	15126-L16897	Exon 11	3191-3192	AACTTCTCACAC-AGAACTGGGACC	1.0 kb
402	15121-L16892	Exon 13	3560-3561	TTGAACAATATA-GTGAAAACATAA	1.6 kb
319	15116-L16887	Exon 14	3674-3675	TATGTTACCTGA-TCCACCATTACC	20.4 kb
292	15114-L16885	Exon 18	7333-7334	ATCCAACAGCAA-TACCAAGCAAAT	6.0 kb
171	15101-L18695	Exon 19	9113-9112, reverse	CCAGGCTTGAAT-CTTGCAGGCAGC	2.0 kb
148	15098-L16869	Exon 20	9260-9261	TCATTCAGAGAA-AATGGAGAGCTA	3.2 kb
265	15111-L16882	Exon 23	9771-9772	ATGTCTGAGCCA-GCGAAATAGGGC	0.7 kb
220	15107-L16878	Exon 24	9960-9961	TAATAGGGAGAT-TCGAGAAGAAAA	5.9 kb
346	15118-L16889	Exon 28	10600-10601	AGAGATAACATG-GAAGAAATCACA	
		stop codon	*10664-10666 (Exon 28)*		

Table 2c. *MCPH1*

Length (nt)	SALSA MLPA probe	*MCPH1* exon[a]	Ligation site NM_024596.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*42-44 (Exon 1)*		
166	15100-L16871	Exon 2	95-96	GTGTGGTCATCC-AATGGAACAGAA	22.1 kb
232	15108-L16879	Exon 4	26 nt before exon 4	GCAAATACTCAT-TAGACTACCTTA	23.7 kb
484	15128-L16899	Exon 9	1910-1911	TTAGATGACTCA-TGTGACGGCTTT	22.4 kb
187	20613-L16874	Exon 10	2004-2005	TCATGACAAGCA-TGCCATCTGAGT	3.1 kb
382	15120-L16891	Exon 11	2038-2039	CGTCATCCAGGT-TGTGGATAAATT	140.4 kb
337	07150-L06762	Exon 13	325 nt before exon 13	TTTAGTGAGAGT-TCAGCCTAGCTA	0.5 kb
436	15124-L16895	Exon 13	2377-2378	CTGTGAACTAGT-CCACCTGTGCGG	23.5 kb
136	15096-L16867	Exon 14	4615-4616	TTGTGCTGACTC-ATGCTGGAGACA	
		stop codon	*2547-2549 (Exon 14)*		

Table 2d. *CDK5RAP2*

Length (nt)	SALSA MLPA probe	*CDK5RAP2* exon[a]	Ligation site NM_018249.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*190-192 (Exon 1)*		
328	15117-L16888	Exon 3	342-343	TCAGAAGAAACA-GTGTCTCCCACC	43.3 kb
159	15099-L18787	Exon 11	1217-1218	TGAAATTGAAAA-GCTCAGTGCTGC	37.7 kb
177 «	16144-L16873	Exon 14	1758-1759	AGTGAAGGCTTA-ATAACAGAAAAG	39.4 kb
244	15110-L16881	Exon 22	3124-3125	TGAAGGAGTTTA-AAACTTGTAATA	39.6 kb
142	15097-L16868	Exon 31	4883-4884	GAAGGCGTATGA-GAAGCTGGATGA	7.5 kb
195	15104-L16875	Exon 35	5528-5529	GAGCAAGTTTGT-GAGCAGTGTGAG	
		stop codon	*5869-5871 (Exon 38)*		

Table 2e. *CENPJ*

Length (nt)	SALSA MLPA probe	*CENPJ* exon[a]	Ligation site NM_018451.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*168-170 (Exon 2)*		
310	15115-L16886	Exon 2	262-263	GGTCATATTAAA-TCGTGGATTTCC	2.9 kb
208 #	15106-L16877	Exon 4	789-790	CTCCAGGAAATA-CCACCACTGGAG	3.3 kb
416	15122-L16893	Exon 7	1407-1408	AGCCGCTGTTTA-AAATGGATAGAC	21.4 kb
472	15127-L16898	Exon 13	3560-3561	GAGCCACTTGAA-CCACTGAACTTC	
		stop codon	4182-4184 (Exon 17)		

Table 2f. *WDR62*

Length (nt)	SALSA MLPA probe	*WDR62* exon[a]	Ligation site NM_001083961.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*76-78 (Exon 1)*		
154	16812-L19467	Exon 1	112-113	ATGCGCGGAACG-ATGCAGGGGAGA	
		stop codon	*4645-4647 (Exon 32)*		

[a] See section Exon numbering on page 1 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.
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.

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

- Mahmood S et al (2011). Autosomal Recessive Primary Microcephaly (MCPH): clinical manifestations, genetic heterogeneity and mutation continuum. *Orphanet J Rare Dis*. 6:39
- 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.
- Thornton GK, Woods CG (2009). Primary microcephaly: do all roads lead to Rome? *Trends genet* 25:501-10.
- 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 P355 Microcephaly

- Caraffi SG et al. (2022). MCPH1: A Novel Case Report and a Review of the Literature. *Genes*, 13(4), 634.

P355 product history	
Version	*Modification*
B1	One probe targeting the *MCPH1* gene has been removed from the probemix.
A2	One reference probe has been replaced.
A1	First release.

Implemented changes in the product description
Version B1-02 – 26 November 2024 (04P) - The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate. *Version B1-01 – 24 May 2022 (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 *MCPH1*, *WDR62*, *CDK5RAP2*, *ASPM*, and *CENPJ* genes updated according to new version of the NM_ reference sequence. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. *Version A2-01 – 25 July 2018 (01P)* - Product description restructured and adapted to a new template. - Exon numbering of the exon 14 probe for the *MCPH1* gene has been changed.

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