Product Description SALSA® MLPA® probemix P046-C1 TSC2

To be used with the MLPA General Protocol.

Version C1. For complete product history see page 9.

Catalogue numbers:
- **P046-025R:** SALSA MLPA Probemix P046 TSC2, 25 reactions.
- **P046-050R:** SALSA MLPA Probemix P046 TSC2, 50 reactions.
- **P046-100R:** SALSA MLPA Probemix P046 TSC2, 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 P046 TSC2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the human *TSC2* gene, which is associated with tuberous sclerosis complex.

Tuberous sclerosis complex (TSC) is a genetic disorder characterized by abnormalities of the skin, brain, kidney, heart and lungs, seizures, and intellectual disability/developmental delay. Central nervous system tumours are the leading cause of morbidity and mortality; renal disease is the second leading cause of early death. The diagnosis of TSC is based on clinical findings and affects approximately 1 in 6.000 live births. TSC is inherited in an autosomal dominant manner and caused by mutations in either the *TSC1* or *TSC2* gene.

TSC2 mutations account for the majority (51%–82%) of all TSC patients as compared to *TSC1* mutations (~24%) (Sancak et al. 2005). *TSC2* mutations appear to be more common in sporadic TSC cases, while inherited cases result nearly equal from *TSC1* and *TSC2* mutations. Presently, more than 450 different disease-causing mutations are known for *TSC1* and more than 1300 are known for *TSC2*. Truncating mutations are the most common mutation type in the *TSC1* (80%) and in the *TSC2* (65%) gene. Large genomic deletions are rare in *TSC1* (3%), but occur more frequently in the *TSC2* gene (6%) (Mayer et al. 2014).

More information is available on https://www.ncbi.nlm.nih.gov/books/NBK1220/.

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 P046-C1 TSC2 contains 52 probes with amplification products between 130 and 490 nt. This includes one probe for each of the 42 exons of the *TSC2* gene and one probe for the *PKD1* gene, downstream of *TSC2*. In addition, 8 reference probes are included and detect 8 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, 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.mpla.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 5 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 from peripheral blood, 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 TSC. 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) 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 number NA04520 from the Coriell Institute has been tested at MRC-Holland and can be used as a positive control sample to detect a deletion of *TSC2* exons 1-15. 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 or in or near the *TSC2* 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 (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 in some cases 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:

- In most populations, the major cause of genetic defects in the *TSC2* gene are small (point) mutations, none of which will not be detected by using SALSA MLPA probemix P046 TSC2.
- 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 one or more than one consecutive probe (Table 2) should be confirmed by SALSA MLPA probemix P337 TSC2 or 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.

Tuberous sclerosis mutation database:
http://chromium.lovd.nl/LOVD2/TSC/home.php?select_db=TSC2. We strongly encourage users to deposit positive results in the Tuberous sclerosis mutation 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 SNPs and unusual results (e.g., a duplication of *TSC2* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA P046-C1 TSC2 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) reference	TSC2
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
136	**TSC2 probe** 16725-L19337		**Exon 28**
142	**TSC2 probe** 01819-L20598		**Exon 2**
148	**TSC2 probe** 01820-L20599		**Exon 3**
154 «	**TSC2 probe** 03171-L20600		**Exon 37**
160	**TSC2 probe** 01822-L01387		**Exon 5**
166	**TSC2 probe** 03170-L10852		**Exon 29**
172	**TSC2 probe** 04025-L10853		**Exon 10**
178	Reference probe 02330-L01818	12q23	
184	**TSC2 probe** 02350-L20601		**Exon 9**
190	**TSC2 probe** 16727-L19338		**Exon 24**
196	**TSC2 probe** 04024-L10855		**Exon 6**
202 «	**TSC2 probe** 16729-L19340		**Exon 40**
208	**TSC2 probe** 01826-L20602		**Exon 11**
214 «	**TSC2 probe** 16730-L19341		**Exon 33**
221	**TSC2 probe** 03169-L20603		**Exon 25**
227	**TSC2 probe** 01827-L20604		**Exon 12**
232	**TSC2 probe** 01828-L20605		**Exon 14**
241	Reference probe 16329-L19069	7p14	
250	**TSC2 probe** 10581-L20606		**Exon 15**
256	**TSC2 probe** 10526-L11086		**Exon 17**
265 « ¬	**PKD1 probe** 11192-L11126		**Downstream TSC2**
273 «	**TSC2 probe** 11191-L11125		**Exon 32**
281	**TSC2 probe** 01832-L01397		**Exon 19**
286	Reference probe 15880-L18438	2p16	
293 « Ж	**TSC2 probe** 16731-SP0383-L20607		**Exon 35**
301	**TSC2 probe** 16732-L20608		**Exon 21**
313	**TSC2 probe** 01834-L20609		**Exon 22**
325	**TSC2 probe** 17204-L19345		**Exon 16**
334	**TSC2 probe** 01835-L20613		**Exon 23**
339 Ж	**TSC2 probe** 16735-SP0385-L20963		**Exon 13**
346	**TSC2 probe** 03166-L02569		**Exon 18**
352	**TSC2 probe** 16736-L19347		**Exon 26**
358 « Ж	**TSC2 probe** 16737-SP0386-L19348		**Exon 41**
364	Reference probe 15897-L18094	3p21	
371	**TSC2 probe** 01838-L20615		**Exon 27**
378	**TSC2 probe** 10527-L20616		**Exon 20**
382	Reference probe 16909-L20513	17q12	
390	**TSC2 probe** 01839-L20617		**Exon 30**
397	**TSC2 probe** 01840-L20618		**Exon 31**
402	**TSC2 probe** 16738-L20619		**Exon 8**
409 «	**TSC2 probe** 02356-L02220		**Exon 34**
418 «	**TSC2 probe** 01842-L01407		**Exon 36**
427	**TSC2 probe** 16740-L21305		**Exon 1**
434 Ж	**TSC2 probe** 17355-SP0496-L20969		**Exon 4**
445 «	**TSC2 probe** 02445-L01409		**Exon 42**
452 «	**TSC2 probe** 01843-L18983		**Exon 38**
460	**TSC2 probe** 16741-L19352		**Exon 7**
466	Reference probe 05171-L04552	13q12	
472 «	**TSC2 probe** 15654-L12745		**Exon 39**
481 Ж	**TSC2 probe** 17356-SP0497-L20970		**Exon 4**
490	Reference probe 06887-L21592	8p23	

« 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 P046-C1 TSC2 product description is the exon numbering from the RefSeq transcript NM_000548.3, which is identical to the LRG_487 sequence. The exon numbering and NM sequence used is from 01/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. TSC2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	TSC2 Exon	Ligation site NM_000548.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*107-109 (exon 2)*		
427	16740-L21305	Exon 1	112 nt after exon 1	ACTGCAACCCGA-CTCCGGAGCTCC	0.5 kb
142	01819-L20598	Exon 2	133-134	AGCAAAGATTCA-GGCTTGAAGGAG	1.8 kb
148	01820-L20599	Exon 3	289-290	ATCCGGATGATA-GGGCAGATTTGT	3.1 kb
434 Ж	17355-SP0496-L20969	Exon 4	86 nt; 113 nt after exon 4	TTGGACTCCTGC-27 nt spanning oligo-AGGGGCTGATGG	0.1 kb
481 Ж	17356-SP0497-L20970	Exon 4	187 nt; 217 nt after exon 4	TGACCGTAGGCA-30 nt spanning oligo-TGCACCTTCCTC	0.7 kb
160	01822-L01387	Exon 5	478-479	GCCCTCTTCTTT-AAGGTCATCAAG	1.1 kb
196	04024-L10855	Exon 6	616-617	TGGATGGATGTT-GGCTTGTCCTCG	0.9 kb
460	16741-L19352	Exon 7	34 nt after exon 7	GCCGGCCCATTT-CACCCTGGTTTC	0.4 kb
402	16738-L20619	Exon 8	791-792	TGGTCTGCTACA-ACTGCCTGCCGG	0.5 kb
184	02350-L20601	Exon 9	933-934	CATCTACAACAT-GTGCCACCTCAT	1.7 kb
172	04025-L10853	Exon 10	1035-1034 reverse	TCCTGAGAGAAT-AGAGCCGGTGGG	1.9 kb
208	01826-L20602	Exon 11	1116-1117	GGTGTCCTATGA-GATCGTCCTGTC	1.2 kb
227	01827-L20604	Exon 12	1268-1269	TCCATGACCTGT-TGACCACGGTGG	0.5 kb
339 Ж	16735-SP0385-L20963	Exon 13	73 nt; 46 nt before exon 13	AAACCAGCCTCT-27 nt spanning oligo-GAGCGCCGGAGG	0.6 kb
232	01828-L20605	Exon 14	1521-1522	GCTGTCCTTTGT-GCTGCTCATCAA	1.3 kb
250	10581-L20606	Exon 15	1561-1562	GAGGAGCTGATT-AACTCAGTGGTC	1.3 kb
325	17204-L19345	Exon 16	1751-1752	TGGAAGAAAGGG-ATGTGGCCGCAT	5.0 kb
256	10526-L11086	Exon 17	1890-1891	CAGCCACATTCA-GCTCCACTACAA	1.1 kb
346	03166-L02569	Exon 18	2010-2011	GCCCAACAAGGA-TGGAGTCGTGCG	0.2 kb
281	01832-L01397	Exon 19	2080-2081	TCTGAGAAGAAG-ACCAGCGGCCCC	0.5 kb
378	10527-L20616	Exon 20	2232-2233	GCTGAAGCTGGT-TCTGGGCAGGCT	0.6 kb
301	16732-L20608	Exon 21	2349-2350	AAAGACACTGGA-GCGGCTCCGAGG	1.5 kb
313	01834-L20609	Exon 22	2608-2609	ACGCACATCTCA-GCCACAGCCAGC	1.5 kb
334	01835-L20613	Exon 23	2711-2712	ATGCCAGTGTGT-TCGCCATCTCCC	0.2 kb
190	16727-L19338	Exon 24	2778-2779	TCTGGCCCATCA-CGTCATAGCCAT	0.5 kb
221	03169-L20603	Exon 25	2928-2927 reverse	TGGGTCTCTCGT-TGAGACTAGTAC	1.1 kb
352	16736-L19347	Exon 26	3025-3026	TCTGCAGCCGAG-GCCTTCCGGTGC	1.4 kb
371	01838-L20615	Exon 27	3146-3147	AGGCTGACGATA-GCCTGAAAAACC	0.2 kb
136	16725-L19337	Exon 28	3317-3318	TCACTGTGACGA-CAAGCGTGGGAA	0.2 kb
166	03170-L10852	Exon 29	3415-3416	GTGCATGTGAGA-CAGACCAAGGAG	0.7 kb
390	01839-L20617	Exon 30	3609-3610	GAAACCTGAGAA-GGCCTCAGCTGG	1.5 kb
397	01840-L20618	Exon 31	3858-3859	AGCCCTGTACAA-GTCACTGTCGGT	0.8 kb
273 «	11191-L11125	Exon 32	3979-3980	CACAGGAGCGTT-TCCTGGGCAGGT	1.3 kb
214 «	16730-L19341	Exon 33	4063-4064	GGGTTGGAGGAC-GTTGAGGCAGCG	0.9 kb
409 «	02356-L02220	Exon 34	4535-4536	AGAGAGTAGAGA-GGGACGCCTTAA	0.2 kb
293 « Ж	16731-SP0383-L20607	Exon 35	49 nt; 25 nt before exon 35	TGGGCTGTGGCT-24 nt spanning oligo-TGCCCACCATCC	0.4 kb
418 «	01842-L01407	Exon 36	4724-4725	AGATCCCATCAT-ACGACACCCACA	1.0 kb
154 «	03171-L20600	Exon 37	4883-4884	CGGACAAGGTGT-ACCTGGGAGGCC	0.5 kb
452 «	01843-L18983	Exon 38	5041-5042	GGCAACGACTTT-GTGTCCATTGTC	1.0 kb
472 «	15654-L12745	Exon 39	35 nt before exon 39	TCAGCACACGCT-GTGTGCGGGGAT	0.2 kb
202 «	16729-L19340	Exon 40	5194-5195	GGCCTTGTGGAC-ACCAGCGTGGCC	0.3 kb
358 « Ж	16737-SP0386-L19348	Exon 41	44 nt; 20 nt after exon 41 reverse	CGCCCCACAGCT-24 nt spanning oligo-GGGAGCCCCATA	0.2 kb
445 «	02445-L01409	Exon 42	5470-5471	ACACCTGGCTAT-GAGGTGGGCCAG	0.9 kb
		stop codon	*5528-5530 (exon 42)*		
265 « ¬	11192-L11126	PKD1	Exon 46	ACGGTTTCTAGC-CTCTGAGATGCT	

« 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 P046-C1 TSC2 product description is the exon numbering from the RefSeq transcript NM_000548.3, which is identical to the LRG_487 sequence. The exon numbering and NM sequence used is from 01/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P337 TSC2: Contains more TSC2 probes. This probemix can be used for confirmation of obtained P046 results.
- P124 TSC1: Characterisation of TSC1 deletions/duplications.
- P351/P352 PKD1-PKD2: These probemixes contain probes for the PKD1 and PKD2 genes.

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P046 Product history	
Version	*Modification*
C1	The missing exons of TSC2 have been added and several target and reference probes have been replaced.
B3	The 88 and 96 nt control fragments have been replaced.
B2	Small change in length / peak height of probes, but no change in sequence detected.
A1	First release.

Implemented changes in the product description

Version C1-01 – 22 February 2018 (01P)
- Product description restructured and adapted to a new template.

Version 13 – 10 February 2017 (55)
- Warning added below Figure 1: A non-specific peak can occur at 450 nt in the no DNA sample.
- Minor textual changes on page 2 and page 5.

Version 12 – 26 November 2015 (55)
- Product description adapted to a new lot (lot number changed, small changes in Table 1 and Table 2, new picture included).

Version 11 - 26 March 2015 (54)
- Exon numbering has been adjusted according to NM_000548.3

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