Product Description
SALSA® MLPA® Probemix P046-D1 TSC2

To be used with the MLPA General Protocol.

Version D1
As compared to version C1, the length of several probes has been adjusted and four reference probes have been replaced and one reference probe has been added. Multiple target probes targeting intronic sequences have been replaced by target probes targeting exonic sequences which were previously present in P337-B1. In addition, one flanking probe has been replaced. For complete product history see page 10.

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 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.

Intended purpose
The SALSA MLPA Probemix P046 TSC2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *TSC2* gene in genomic DNA isolated from human peripheral whole blood specimens. P046 TSC2 is intended to confirm a potential cause for and clinical diagnosis of tuberous sclerosis complex (TSC) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P046 TSC2 should be confirmed by using SALSA MLPA probemix P337 TSC2 Confirmation assay or with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *TSC2* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g. from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1]Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of the product description. In all other countries, the product is for research use only (RUO).
[2]To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background

Tuberous sclerosis complex (TSC) is a genetic disorder characterised by seizures and intellectual disability/developmental delay, and by abnormalities of the skin, brain, kidney, heart, and lungs. 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 6000 live births worldwide. Prevalence is estimated to be 1 in 11.300-25.000 in Europe. TSC is inherited in an autosomal dominant manner and is caused by mutations in either the *TSC1* or *TSC2* genes.

TSC2 mutations account for the majority (~69%) of all TSC patients as compared to *TSC1* mutations (~26%). *TSC2* mutations appear to be more common in sporadic TSC cases, while inherited cases result from *TSC1* and *TSC2* mutations in a nearly equal proportion. 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 the *TSC2* (65%) genes. Large genomic deletions are rare in *TSC1* (3%), but occur more frequently in the *TSC2* gene (5%). The frequency of somatic mosaicism for large deletions and duplications in the *TSC1* and *TSC2* genes in affected individuals with TSC has been reported as ~5% (Kozlowski et al. 2007) up to 10-25% (Jang et al. 2012). Cases of mosaic partial *TSC2* gene deletions have been reported (Sampson et al. 1997; Verhoef et al. 1999).

Some affected individuals have features of both TSC, caused by deletion of *TSC2*, and autosomal dominant polycystic kidney disease (ADPKD), caused by deletion of *PKD1*. Individuals with the TSC2/PKD1 contiguous gene deletion syndrome are also at risk of developing the complications of ADPKD, which include cystic lesions in other organs (e.g., the liver).

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

Gene structure

The *TSC2* gene spans ~41 kilobases (kb) on chromosome 16p13 and contains 42 exons. The *TSC2* LRG_487 is available at www.lrg-sequence.org and is identical to GenBank NG_005895.1.

Transcript variants

For *TSC2,* multiple variants have been described. Transcript variant 1 is the most predominant and encodes isoform 1 (NM_000548.5; 6415 nt; coding sequence 111-5534; http://www.ncbi.nlm.nih.gov/gene/7249). The ATG translation start site is located in exon 2 and the stop codon is located in exon 42.

Exon numbering

The *TSC2* exon numbering used in this P046-D1 TSC2 product description is the exon numbering from the LRG_487 sequence. 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 LRG 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 P046-D1 TSC2 contains 52 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 42 probes for *TSC2* and one flanking probe. 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 from human peripheral whole blood specimens, 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 TSC. 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 NA04520 from the Coriell Institute has been tested with this P046-D1 probemix at MRC Holland and can be used as a positive control sample to detect a heterozygous deletion of *TSC2* exons 1-15. Sample ID number NA06226 can be used as a positive control sample to detect a homozygous duplication of all target probes. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics
TSC2 deletions and duplications account for ~5% of all TSC cases. The analytical sensitivity and specificity for the detection of deletions or duplications in the *TSC2* gene is very high and can be considered >99% (based on a 2005-2021 literature study performed at MRC Holland).

Analytical performance can be compromised by: Single-nucleotide variants (SNVs) or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for the probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). 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 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 (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.

P046 specific note:

- The use of the fixed cut-off values for the FR of the probes as mentioned in the table above will not allow the detection of deletions/duplications in all samples that are a mixture of normal and abnormal cells, such as mosaic samples. Mosaicism is known to occur in ~5% (Kozlowski et al. 2007) up to 10-25% (Jang et al. 2012) of TSC patients. According to literature, MLPA should be able to detect a deletion when it is present in at least 30% of the cells, and a duplication when it is present in at least 40% of the cells (Consugar et al. 2008; van Veghel-Plandsoen et al. 2011). In order to detect mosaic samples the analysis needs to have little amounts of variation and the ratios should be significantly different from the reference samples (see Coffalyser.Net Reference Manual, Appendix I – Normalisation and result interpretation).

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *TSC2* are small (point) mutations, none of which will 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. 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 with the P046 TSC2 probemix must be confirmed. The SALSA MLPA probemix P337 TSC2 Confirmation can be used for initial confirmation of results. Alternatively, copy number changes can 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.

Copy number changes detected by only a single probe always require confirmation with the P337 TSC2 Confirmation probemix or 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.

Leiden Open Variation Database (LOVD) for *TSC2*

https://databases.lovd.nl/shared/genes/TSC2. We strongly encourage users to deposit positive results in the LOVD for the *TSC2* gene. 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 (e.g., a duplication of *TSC2* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P046-D1 TSC2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*TSC2*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q	
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** 22266-L31542		**Exon 31**
166 ¥	**TSC2 probe** 01822-L31380		**Exon 5**
172 ¥ «	**TSC2 probe** 03170-L31381		**Exon 29**
178 *	Reference probe 05458-L04861	22q	
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**
239 ¥	**TSC2 probe** 01828-L31537		**Exon 14**
244 ¥	Reference probe 16329-L30925	7p	
250	**TSC2 probe** 10581-L20606		**Exon 15**
257 *	**TSC2 probe** 22270-L31391		**Exon 4**
265 ¥	**TSC2 probe** 10526-L31384		**Exon 17**
274 ¥ «	**TSC2 probe** 11191-L31385		**Exon 32**
281 «	**TSC2 probe** 01832-L01397		**Exon 19**
288 ¥	Reference probe 15880-L30312	2p	
296 * «	**TSC2 probe** 22262-L31543		**Exon 35**
304 ¥ «	**TSC2 probe** 16732-L30314		**Exon 21**
310 * ¬ « Ж	PKD1 probe 22264-L27363		downstream
319 *	Reference probe 09767-L10182	15q	
325	**TSC2 probe** 17204-L19345		**Exon 16**
334 «	**TSC2 probe** 01835-L20613		**Exon 23**
338 *	**TSC2 probe** 22272-L31393		**Exon 13**
346 * «	**TSC2 probe** 16723-L19335		**Exon 39**
352 «	**TSC2 probe** 16736-L19347		**Exon 26**
358 ¥ «	**TSC2 probe** 03166-L31394		**Exon 18**
364 * «	**TSC2 probe** 11935-L12755		**Exon 41**
371 «	**TSC2 probe** 01838-L20615		**Exon 27**
378 * «	**TSC2 probe** 21363-L30216		**Exon 20**
385 *	Reference probe 18677-L30318	11p	
395 ¥ «	**TSC2 probe** 01839-L31534		**Exon 30**
402	**TSC2 probe** 16738-L20619		**Exon 8**
409 * «	**TSC2 probe** 22274-L31396		**Exon 34**
418 *	Reference probe 21261-L29869	20p	
428 * «	**TSC2 probe** 22275-L31397		**Exon 36**
436 ¥	**TSC2 probe** 16740-L31544		**Exon 1**
442 * «	**TSC2 probe** 22263-L20609		**Exon 22**
453 ¥ «	**TSC2 probe** 01843-L30689		**Exon 38**
460 * « ±	**TSC2 probe** 22276-L31399		**Exon 42**
466	Reference probe 05171-L04552	13q	
475 *	**TSC2 probe** 22278-L31400		**Exon 7**
489 *	**TSC2 probe** 22260-L31382		**Exon 10**
500 *	Reference probe 09682-L22509	3p	

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

* New in version D1.
¥ Changed in version D1. 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.
± SNP rs45517416 influences the 460 nt probe signal (22276-L31399). SNP rs766472320 could influence the 202 nt probe signal (16729-L19340). In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.
Ж The PKD1 probe is more sensitive to denaturation problems than the TSC2 probes. A low signal of the PKD1 probe can be found together with normal signals for the TSC2 probes.
¬ 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. *TSC2* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*TSC2* exon[a]	Ligation site NM_000548.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
436	16740-L31544	Exon 1	113 nt after exon 1	ACTGCAACCCGA-CTCCGGAGCTCC	0.5 kb
		start codon	*111-113 (Exon 2)*		
142 «	01819-L20598	Exon 2	137-138	AGCAAAGATTCA-GGCTTGAAGGAG	1.8 kb
148	01820-L20599	Exon 3	293-294	ATCCGGATGATA-GGGCAGATTTGT	3.2 kb
257	22270-L31391	Exon 4	217 nt after exon 4	ACAGATGGTCAC-TGCACCTTCCTC	0.7 kb
166	01822-L31380	Exon 5	482-483	GCCCTCTTCTTT-AAGGTCATCAAG	1.1 kb
196	04024-L10855	Exon 6	620-621	TGGATGGATGTT-GGCTTGTCCTCG	0.8 kb
475	22278-L31400	Exon 7	724-725	GATCTGTCTGCT-GTGCGTCCGGAC	0.5 kb
402	16738-L20619	Exon 8	795-796	TGGTCTGCTACA-ACTGCCTGCCGG	0.5 kb
184	02350-L20601	Exon 9	937-938	CATCTACAACAT-GTGCCACCTCAT	1.7 kb
489	22260-L31382	Exon 10	1039-1038 reverse	TCCTGAGAGAAT-AGAGCCGGTGGG	1.9 kb
208	01826-L20602	Exon 11	1120-1121	GGTGTCCTATGA-GATCGTCCTGTC	1.2 kb
227	01827-L20604	Exon 12	1272-1273	TCCATGACCTGT-TGACCACGGTGG	0.7 kb
338	22272-L31393	Exon 13	1467-1468	TGGAGAGATTCT-TCAGGTAGGGGG	0.4 kb
239	01828-L31537	Exon 14	1525-1526	GCTGTCCTTTGT-GCTGCTCATCAA	1.3 kb
250	10581-L20606	Exon 15	1565-1566	GAGGAGCTGATT-AACTCAGTGGTC	1.3 kb
325	17204-L19345	Exon 16	1755-1756	TGGAAGAAAGGG-ATGTGGCCGCAT	5.0 kb
265	10526-L31384	Exon 17	1894-1895	CAGCCACATTCA-GCTCCACTACAA	1.1 kb
358 «	03166-L31394	Exon 18	2014-2015	GCCCAACAAGGA-TGGAGTCGTGCG	0.2 kb
281 «	01832-L01397	Exon 19	2084-2085	TCTGAGAAGAAG-ACCAGCGGCCCC	0.5 kb
378 «	21363-L30216	Exon 20	2236-2237	GCTGAAGCTGGT-TCTGGGCAGGCT	0.6 kb
304 «	16732-L30314	Exon 21	2353-2354	AAAGACACTGGA-GCGGCTCCGAGG	1.5 kb
442 «	22263-L20609	Exon 22	2612-2613	ACGCACATCTCA-GCCACAGCCAGC	1.5 kb
334 «	01835-L20613	Exon 23	2715-2716	ATGCCAGTGTGT-TCGCCATCTCCC	0.2 kb
190 «	16727-L19338	Exon 24	2782-2783	TCTGGCCCATCA-CGTCATAGCCAT	0.5 kb
221 «	03169-L20603	Exon 25	2932-2931 reverse	TGGGTCTCTCGT-TGAGACTAGTAC	1.1 kb
352 «	16736-L19347	Exon 26	3029-3030	TCTGCAGCCGAG-GCCTTCCGGTGC	1.4 kb
371 «	01838-L20615	Exon 27	3150-3151	AGGCTGACGATA-GCCTGAAAAACC	0.2 kb
136 «	16725-L19337	Exon 28	3321-3322	TCACTGTGACGA-CAAGCGTGGGAA	0.2 kb
172 «	03170-L31381	Exon 29	3419-3420	GTGCATGTGAGA-CAGACCAAGGAG	0.7 kb
395 «	01839-L31534	Exon 30	3613-3614	GAAACCTGAGAA-GGCCTCAGCTGG	1.5 kb
160 «	22266-L31542	Exon 31	3862-3863	AGCCCTGTACAA-GTCACTGTCGGT	0.8 kb
274 «	11191-L31385	Exon 32	3983-3984	CACAGGAGCGTT-TCCTGGGCAGGT	1.3 kb
214 «	16730-L19341	Exon 33	4067-4068	GGGTTGGAGGAC-GTTGAGGCAGCG	0.9 kb
409 «	22274-L31396	Exon 34	4551-4552	GGGACGCCTTAA-AGAGCAGAGCCA	0.4 kb
296 «	22262-L31543	Exon 35	4679-4680	CTGCCCAATGAG-GTAGGCGTGGCC	0.3 kb
428 «	22275-L31397	Exon 36	4728-4729	AGATCCCATCAT-ACGACACCCACA	1.0 kb
154 «	03171-L20600	Exon 37	4887-4888	CGGACAAGGTGT-ACCTGGGAGGCC	0.5 kb
453 «	01843-L30689	Exon 38	5045-5046	GGCAACGACTTT-GTGTCCATTGTC	1.1 kb
346 «	16723-L19335	Exon 39	5114-5115	CAGTTCAACTTT-GTCCACGTGATC	0.2 kb
202 « ±	16729-L19340	Exon 40	5198-5199	GGCCTTGTGGAC-ACCAGCGTGGCC	0.3 kb

Length (nt)	SALSA MLPA probe	*TSC2* exon[a]	Ligation site NM_000548.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
364 «	11935-L12755	Exon 41	5350-5351	GCTCCGCCACAT-CAAGCGGCTCCG	0.1 kb
460 « ±	22276-L31399	Exon 42	5371-5372	CTGCCTTCAGAT-CTGCGAGGAAGC	2.3 kb
		stop codon	*5532-5534 (Exon 42)*		
310 ¬ « Ж	22264-L27363	*PKD1* gene	NM_001009944.3; 12250-12251	CCAGTGGTCCGT-CTTTGGCAAGAC	

[a] See section Exon numbering on page 2 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.
± SNP rs45517416 influences the 460 nt probe signal (22276-L31399). SNP rs766472320 could influence the 202 nt probe signal (16729-L19340). In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.
Ж The PKD1 probe is more sensitive to denaturation problems than the TSC2 probes. A low signal of the PKD1 probe can be found together with normal signals for the TSC2 probes.
¬ 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

- P337 TSC2 Confirmation: P337 (version C1) can be used for confirmation of results obtained from P046 TSC2 (version D1). Contains more *TSC2* probes with their ligation sites outside the exon. The ligation sites of *TSC2* probes in P046-D1 are different from probes in P337-C1.
- P124 TSC1: Characterisation of *TSC1* deletions/duplications.
- P351/P352 PKD1-PKD2: These probemixes contain probes for the *PKD1* and *PKD2* genes.

References

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P046 product history	
Version	*Modification*
D1	The length of several probes has been adjusted and four reference probes have been replaced and one reference probe has been added. Multiple target probes targeting intronic sequences have been replaced by target probes targeting exonic sequences which were previously present in P337-B1. In addition, one flanking probe has been replaced.
C1	The missing exons of *TSC2* have been added and several target and reference probes have been replaced.
B3	The 88 and 96 nt DNA Denaturation control fragments have been replaced (QDX2).
B2	Small change in length / peak height, but no change in sequence detected.
B1	Two TSC2 probes and the PKD1 probe have been replaced. In addition, two extra TSC2 probes (exons 16 & 31) have been included. All reference probes have been replaced.
A2	Contains D-control fragments. All control probes have been replaced. No change in the sequences detected by the TSC2 probes, but many TSC2 probes have a different relative peak height and a slightly different amplification length.
A1	Small changes have been made to certain probes in order to reduce the signal of probes with a very high signal. These changes have no effect on the sequence detected.
A0	First release.

Implemented changes in the product description
Version D1-03 – 01 July 2022 (04P) - Warnings added to Table 1 and 2 for salt sensitivity of probes 01819-L20598, 03166-L31394, 01832-L01397, 21363-L30216 and 16732-L30314. - Added ligation site of probe 22264-L27363 in Table 2. - Wording of the 'Confirmation of results' section slightly changed to include the recommendation to confirm results by the P337 TSC2 Confirmation probemix. - New references added. Version D1-02 – 08 April 2021(04P) - Product description rewritten and adapted to a new template. - Wording of the intended purpose has been updated to the new template. The content of the intended purpose did not change. - Numbers and percentages in the clinical background section updated according to recent literature. - Information about positive control sample NA06226 added. - Added note with information about mosaicism in the interpretation of results section. - UK has been added to the list of countries in Europe that accept the CE mark. - Warnings added to Table 1 and 2 for the effect of SNPs rs45517416 and rs766472320 on the probe ratio of probes 22276-L31399 and 16729-L19340, respectively. - Warnings added to Table 1 and 2 for salt sensitivity of probes 22263-L20609, 01835-L20613 and 16727-L19338. - Warning added to Table 1 and 2 for sensitivity to denaturation of probe 22264-L27363. - New references added.

Version D1-01 — 09 October 2019 (02P)
- Product description rewritten and adapted to a new template.
- P046-D1 is now CE marked.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Various minor textual changes.
-Ligation sites of the probes targeting the *TSC2* gene updated according to new version of the NM_ reference sequence.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
Version C1-01 – 22 February 2018 (01P)
- Product description restructured and adapted to a new template.

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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.