Product Description
SALSA® MLPA® Probemix P337-C1 TSC2 Confirmation

To be used with the MLPA General Protocol.

Version C1
As compared to version B1, multiple target probes have been replaced. The length of several probes has been adjusted and five reference probes have been replaced and one reference probe has been added. In addition, one flanking probe has been removed and one has been replaced. For complete product history see page 10.

Catalogue numbers:
- **P337-025R:** SALSA MLPA Probemix P337 TSC2 Confirmation, 25 reactions.
- **P337-050R:** SALSA MLPA Probemix P337 TSC2 Confirmation, 50 reactions.
- **P337-100R:** SALSA MLPA Probemix P337 TSC2 Confirmation, 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 P337 TSC2 Confirmation is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the confirmation of deletions or duplications in the *TSC2* gene as initially observed using the SALSA MLPA P046 TSC2 probemix, in order to confirm a potential cause for and clinical diagnosis of tuberous sclerosis complex (TSC) and for molecular genetic testing of at-risk family members. This assay is for use with genomic DNA isolated from human peripheral whole blood specimens.

Discordant results between the P337 TSC2 Confirmation probemix and the P046 TSC2 probemix should be confirmed by another 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 P337-C1 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 P337-C1 TSC2 Confirmation contains 52 MLPA probes with amplification products between 124 and 500 nucleotides (nt). This includes 42 probes for the *TSC2* gene 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 tuberous sclerosis complex. 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 P337-C1 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 heterozygous 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.

P337 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* gene region are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P337 TSC2 Confirmation.
- 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

The ligation sites of all probes in the P337 TSC2 Confirmation probemix are different as compared to the P046 TSC2 probemix. Detected copy number changes, which are different from those detected with the P046 TSC2 probemix require confirmation by another method, such as long range PCR, qPCR, array CGH or Southern blotting. 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 P337-C1 TSC2 Confirmation

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*TSC2*
64-105	Control fragments – see table in probemix content section for more information		
124	Reference probe 19616-L26275	4p	
130 * «	**TSC2** probe 22292-L31556		**Exon 41**
136 «	**TSC2** probe 11904-L18003		**Exon 38**
142 «	**TSC2** probe 11905-L12726		**Exon 19**
148 *	Reference probe 04445-L03831	4q	
154 * «	**TSC2** probe 22293-L30930		**Exon 29**
160	**TSC2** probe 11908-L12729		**Exon 6**
166 «	**TSC2** probe 11909-L12730		**Exon 24**
172 «	**TSC2** probe 11910-L12731		**Exon 2**
178	Reference probe 10107-L10531	8q	
193 ¥	**TSC2** probe 11911-L31405		**Exon 11**
199 ¥	**TSC2** probe 14105-L31681		**Exon 5**
206 ¥ «	**TSC2** probe 11913-L31408		**Exon 37**
213 ¥ «	**TSC2** probe 11915-L31409		**Exon 33**
219 *	**TSC2** probe 22299-L31415		**Exon 13**
226 * «	**TSC2** probe 22297-L31413		**Exon 32**
232 *	Reference probe 09641-L26943	17q	
238 *	**TSC2** probe 22300-L31421		**Exon 7**
244 «	**TSC2** probe 13543-L12735		**Exon 21**
250	**TSC2** probe 11921-L12742		**Exon 16**
256	**TSC2** probe 11922-L12743		**Exon 8**
263 «	**TSC2** probe 11923-L12744		**Exon 28**
269 * «	**TSC2** probe 22301-L31682		**Exon 39**
275	Reference probe 19811-L19312	9q	
283 *	**TSC2** probe 22271-L31392		**Exon 4**
290 «	**TSC2** probe 11926-L19314		**Exon 26**
296	**TSC2** probe 11927-L19315		**Exon 10**
301 «	**TSC2** probe 11928-L19316		**Exon 27**
309	**TSC2** probe 22302-L31557		**Exon 17**
315 * ¬ « Ж	PKD1 probe 22358-L24695		downstream
322 * «	**TSC2** probe 22261-L31683		**Exon 35**
328 *	Reference probe 21544-L06206	1p	
337 ¥	**TSC2** probe 22306-L31726		**Exon 9**
343 ¥	**TSC2** probe 11932-L31426		**Exon 12**
350 *	**TSC2** probe 22303-L31684		**Exon 15**
357 ¥	**TSC2** probe 13545-L20180		**Exon 3**
365 *	Reference probe 14059-L26885	5q	
373 ¥	**TSC2** probe 11936-L30790		**Exon 14**
384 ¥ «	**TSC2** probe 11937-L30791		**Exon 20**
391 «	**TSC2** probe 13546-L15221		**Exon 30**
401 *	**TSC2** probe 16739-L31799		**Exon 1**
409 * «	**TSC2** probe 22307-L31427		**Exon 31**
418 * «	**TSC2** probe 21790-L31428		**Exon 42**
427 * «	**TSC2** probe 22376-L31429		**Exon 18**
436 * «	**TSC2** probe 22305-L31424		**Exon 36**
445 *	Reference probe 21415-L08695	13q	
453 ¥ «	**TSC2** probe 13550-L31150		**Exon 23**
462 ¥ « ±	**TSC2** probe 11944-L31152		**Exon 25**
472 ¥ «	**TSC2** probe 12803-L31153		**Exon 22**
481 ¥ «	**TSC2** probe 12802-L31154		**Exon 40**
490 ¥ «	**TSC2** probe 16724-L31155		**Exon 34**
500 *	Reference probe 19555-L27674	2p	

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

* New in version C1.
¥ Changed in version C1. 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 rs566118686 influences the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
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
401	16739-L31799	Exon 1	37 nt before exon 1	CTCCCAAGGCGG-CCCTCCGCGCAA	0.8 kb
		start codon	*111-113 (Exon 2)*		
172 «	11910-L12731	Exon 2	224-225	CAGACGGAGTTT-ATCATCACCGCG	1.6 kb
357	13545-L20180	Exon 3	33 nt before exon 3	GTGGCCTGAGCA-CTGGCCCCTTTT	3.2 kb
283	22271-L31392	Exon 4	113 nt after exon 4	GGCACAGGGTCT-AGGGGCTGATGG	0.7 kb
199	14105-L31681	Exon 5	32 nt before exon 5	GCGACGCTGGCA-GGCTCTGCTGAT	1.2 kb
160	11908-L12729	Exon 6	699-700	AGTACATCGCAA-GGATGGTTCAGT	0.7 kb
238	22300-L31421	Exon 7	38 nt before exon 7	CTCGGCCATCCA-GGCAGTGCTGCC	0.6 kb
256	11922-L12743	Exon 8	13 nt after exon 8	TGGGGTTTCTGA-AACTGCTCTGGA	0.3 kb
337	22306-L31726	Exon 9	19 nt before exon 9	GCCAGCCCCTGA-CACGCATTGTGT	1.8 kb
296	11927-L19315	Exon 10	11 nt after exon 10	GGTAAGGCGGTT-TCTGTGTGCAGT	1.8 kb
193	11911-L31405	Exon 11	37 nt before exon 11	AGCAAGCAAGCA-GCTCTGACCCTG	1.3 kb
343	11932-L31426	Exon 12	1349-1350	CTGGTGGAGAGA-TGTGCGGACCAG	0.5 kb
219	22299-L31415	Exon 13	50 nt before exon 13	CCAGTGTGGAGA-AGGAGAGCGCCG	0.7 kb
373	11936-L30790	Exon 14	40 nt after exon 14	GCTCAGGGCTAT-TTCTCCGTGGGC	1.3 kb
350	22303-L31684	Exon 15	1642-1643	GGCCACCCAGTT-GCTGGTGGACCT	1.3 kb
250	11921-L12742	Exon 16	69 nt after exon 16	CTGCATCTGCGT-TGTGTTGGAGTC	4.7 kb
309	22302-L31557	Exon 17	20 nt before exon 17	GCGCCGTGGTGA-GCTGCGTCCTCT	1.1 kb
427 «	22376-L31429	Exon 18	21 nt before exon 18	TGGCTCTGGCTT-TCACCATCCTCT	0.4 kb
142 «	11905-L12726	Exon 19	2202-2203	TGCAGTGCTTGA-AGCAGGTGAGTG	0.3 kb
384 «	11937-L30791	Exon 20	42 nt before exon 20	GCCTCTGTCTCT-AGGGTCCAGAAG	0.7 kb
244 «	13543-L12735	Exon 21	2428-2429	GCTGACAGCATT-AATCTCTTACCA	1.3 kb
472 «	12803-L31153	Exon 22	2539-2540	GGCCTTGTCCAT-CTGCAGCGTGGA	1.5 kb
453 «	13550-L31150	Exon 23	61 nt before exon 23	GAGCAGCCGTGT-TGGCCTTCAGAG	0.4 kb
166 «	11909-L12730	Exon 24	2800-2801	AGCCATGTGGTT-CATCAGGTGCCG	0.4 kb
462 « ±	11944-L31152	Exon 25	7 nt before exon 25	GGTGTGCTCACT-CTGCCAGGGCCT	1.2 kb
290 «	11926-L19314	Exon 26	3061-3062	CAGTGTGTCTGA-ACATGTGGTCCG	1.3 kb
301 «	11928-L19316	Exon 27	3082-3083	CTCCAGCAGGAT-ACAGACGTCCCT	0.3 kb
263 «	11923-L12744	Exon 28	3283-3284	TGGCAGGACCAA-AACCTGGCTGGT	0.3 kb
154 «	22293-L30930	Exon 29	3502-3503	GGTCCGTTCCAT-GTCGGGTGAGCC	0.5 kb
391 «	13546-L15221	Exon 30	34 nt before exon 30	TGCATCAGGTAA-GTGGTGGTCACC	1.7 kb
409 «	22307-L31427	Exon 31	2 nt after exon 31	TCCAACACAGGT-GAGTGGCATGGC	0.7 kb
226 «	22297-L31413	Exon 32	3975-3976	GACAGCTGCACA-GGAGCGTTTCCT	1.3 kb
213 «	11915-L31409	Exon 33	4 nt after exon 33	ACAGCAGGGTGA-GTGTGGCTCAGA	0.4 kb
490 «	16724-L31155	Exon 34	4150-4151	GGAGAAGTCGCT-CCACGCGGAGGA	0.7 kb
322 «	22261-L31683	Exon 35	28 nt before exon 35	AGGCCCTCACCT-GGGTGCCCACCA	0.3 kb
436 «	22305-L31424	Exon 36	22 nt before exon 36	GTCTGGGGCTCA-GGCAGGGCTCTG	1.0 kb
206 «	11913-L31408	Exon 37	4802-4803	ATCCTGTCCAAT-GAGCATGGCTCC	0.5 kb
136 «	11904-L18003	Exon 38	57 nt before exon 38	GCCCCAGTGCAA-GGCACAGAGGGC	1.2 kb
269 «	22301-L31682	Exon 39	35 nt before exon 39	TCAGCACACGCT-GTGTGCGGGGAT	0.2 kb
481 «	12802-L31154	Exon 40	10 nt before exon 40	CGTGACCACCAA-GTCTCCCCAGAC	0.3 kb
130 «	22292-L31556	Exon 41	20 nt after exon 41	ACACCCCGCTGA-GGGAGCCCCATA	0.2 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
418 «	21790-L31428	Exon 42	5474-5475	ACACCTGGCTAT-GAGGTGGGCCAG	5.7 kb
		stop codon	*5532-5534 (Exon 42)*		
315 ¬ « Ж	22358-L24695	*PKD1* gene	NM_001009944.3; 36 nt before exon 35	CTGCCTCCTGGA-GGCCGGGATGAA	

[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 rs566118686 influences the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
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

- P046 TSC2: Contains *TSC2* probes. This probemix should be used for primary screening of *TSC2*. Most probes are located within 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

- Consugar MB et al. (2008). Characterization of large rearrangements in autosomal dominant polycystic kidney disease and the PKD1/TSC2 contiguous gene syndrome. *Kidney Int.* 74(11):1468-79.
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P337 product history	
Version	*Modification*
C1	Multiple *TSC2* probes have been replaced by *TSC2* probes from P046-C1. The length of several probes has been adjusted and five reference probes have been replaced and one reference probe has been added. In addition, one flanking probe has been removed and one has been replaced.
B1	Four *TSC2* probes were added and one replaced, one flanking probe was added. Two reference probes have been replaced and two removed. In addition, the control fragments have been adjusted (QDX2).
A2	Eight probes have a small change in length, no change in sequence detected.
A1	First release.

Implemented changes in the product description

Version C1-04 – 01 July 2022 (04P)
- Warnings for salt sensitivity removed from Table 1 and 2 for probes 11932-L31426 and 22302-L31557.
- Added ligation site of probe 22358-L24695 in Table 2.
- Wording of the 'Confirmation of results' section slightly changed to include the first tier P046 TSC2 probemix.
- New references added.

Version C1-03 – 13 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.
- Warning added to Table 1 and 2 for the effect of SNP rs566118686 on probe ratio of probe 11944-L31152.
- Warnings added to Table 1 and 2 for salt sensitivity of probes 11910-L12731, 11932-L31426, 22302-L31557, 22376-L31429, 11905-L12726, 11937-L30791, 13543-L12735, 12803-L31153, 13550-L31150 and 11909-L12730.
- Warning added to Table 1 and 2 for sensitivity to denaturation of probe 22358-L24695.
- New references added.

Version C1-02 — 22 April 2020 (02P)
- Correction in the intended use section: P337 (version C1 and higher) is intended to be used as confirmation of results obtained by P046 (version **D1** and higher).

Version C1-01 — 09 October 2019 (02P)
- Product description rewritten and adapted to a new template.
- P337-C1 is now CE marked.
- Probemix name changed to TSC2 Confirmation.
- 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).

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	EUROPE*
	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.