MRC Holland SALSA® MLPA®

Instructions for Use
SALSA® MLPA® Probemix P046 TSC2

See also the MLPA General Protocol, and the product description of the SALSA® MLPA® Reagent Kit and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P046 TSC2 product page on our website to find Certificates of Analysis and a list of related products.

Product name	**SALSA® MLPA® Probemix P046 TSC2**
Version	D1
Catalogue numbers	P046-025R (25 reactions) P046-050R (50 reactions) P046-100R (100 reactions)
Basic UDI-DI	872021148P0465U
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	**Catalogue numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE CE 2797 COLOMBIA
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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

Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State in which the user and/or the patient is located.

Changes in this Product Version:
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 Multiple target probes targeting intronic sequences have been replaced by target probes targeting exonic sequences which were previously present in P337-B1.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.

1. Intended Purpose

The SALSA MLPA Probemix P046 TSC2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual 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 IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection method	Standard methods
Extraction method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration (and) treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of TSC.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80-1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*TSC2*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

Study	Description
Expected values for copy numbers in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in the product description can be used. Cut-off values for copy number determination were verified with P046 TSC2 in 108 samples from healthy individuals with normal copy number and two samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.
Limit of detection	A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommended input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P046 TSC2 on two samples with known CNVs and on one sample without any mutation. Only one minor deviation has been observed with one of the positive samples, with two target probes having a slightly higher FR than the cut-off with the use of 250 ng. This is acceptable, since only two probes are affected, which would not lead to a false result, as the result of multiple target probes need to be evaluated together. All other samples gave the expected results using both the lower and upper input amount of DNA. Internal testing was carried out with P046 TSC2 to investigate the effect of mosaicism on probe performance. Mosaicism was simulated by mixing a DNA sample carrying a heterozygous duplication or a heterozygous deletion in *TSC2* with varying amounts of normal DNA, creating mixtures with 30-90% of aberrant DNA. Because FR cut-off values were set using non-mosaic samples, they do not apply directly to mixed samples. In these cases, FR depends on the level of mosaicism and may fall into an ambiguous range. Results showed that when a deletion is present in less than 80% of cells or a duplication in less than 40% of cells, P046 TSC2 often produced false negatives. At 80% or higher for deletions, 40% or higher for duplications, P046 TSC2 reliably detected the deletion/duplication, with most probes showing the expected FRs (≤0.8 for a deletion, ≥1.2 for a duplication).
Interfering substances	SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction. A study using P046 TSC2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs. For most probes, expected FRs

<table>
<tr><td></td><td>(FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.

<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 364/378 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 376/378 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 374/378 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 373/378 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 222/378 measurements</td></tr>
</table>
* Results are summarised for all probes across all three samples tested in triplicate.

Minor effects were seen with all interferents, with exception of hemoglobin which had the largest effect on copy number determination. While the use of other interferents led to ambiguous values only, the use of hemoglobin led to final ratios in the incorrect range.

Importantly, Coffalyser.Net issues warnings for the samples in which the interferent showed an effect, as well as lowered quality scores, which means that the samples need to be re-tested according to the P046 IFU.

To minimise variability across 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.</td></tr>
<tr><td>Cross-reactivity</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, two previously genotyped samples were tested using P046 TSC2 and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 1875/1890 data points, leading to a precision of >99%.</td></tr>
<tr><td>Clinical validity*</td><td>TSC2: Among TSC patients with TSC2 mutations, about 6% of the mutations are large deletions/duplications (CNVs) in TSC2 (Mayer et al. 2014).

*(Based on a 2000-2025 literature review)</td></tr>
</table>

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
16p13.3	*TSC2*	Intron 1 (Exon 1)	0.5 kb	436	16740-L31544	Ø
16p13.3	*TSC2*	Exon 2	1.8 kb	142	01819-L20598	
16p13.3	*TSC2*	Exon 3	3.2 kb	148	01820-L20599	
16p13.3	*TSC2*	Intron 4 (Exon 4)	0.7 kb	257	22270-L31391	Ø
16p13.3	*TSC2*	Exon 5	1.1 kb	166	01822-L31380	
16p13.3	*TSC2*	Exon 6	0.8 kb	196	04024-L10855	
16p13.3	*TSC2*	Exon 7	0.5 kb	475	22278-L31400	
16p13.3	*TSC2*	Exon 8	0.5 kb	402	16738-L20619	
16p13.3	*TSC2*	Exon 9	1.7 kb	184	02350-L20601	
16p13.3	*TSC2*	Exon 10	1.9 kb	489	22260-L31382	
16p13.3	*TSC2*	Exon 11	1.2 kb	208	01826-L20602	
16p13.3	*TSC2*	Exon 12	0.7 kb	227	01827-L20604	
16p13.3	*TSC2*	Exon 13	0.4 kb	338	22272-L31393	
16p13.3	*TSC2*	Exon 14	1.3 kb	239	01828-L31537	
16p13.3	*TSC2*	Exon 15	1.3 kb	250	10581-L20606	
16p13.3	*TSC2*	Exon 16	5.0 kb	325	17204-L19345	
16p13.3	*TSC2*	Exon 17	1.1 kb	265	10526-L31384	
16p13.3	*TSC2*	Exon 18	0.2 kb	358	03166-L31394	
16p13.3	*TSC2*	Exon 19	0.5 kb	281	01832-L01397	
16p13.3	*TSC2*	Exon 20	0.6 kb	378	21363-L30216	
16p13.3	*TSC2*	Exon 21	1.5 kb	304	16732-L30314	
16p13.3	*TSC2*	Exon 22	1.5 kb	442	22263-L20609	
16p13.3	*TSC2*	Exon 23	0.2 kb	334	01835-L20613	
16p13.3	*TSC2*	Exon 24	0.5 kb	190	16727-L19338	
16p13.3	*TSC2*	Exon 25	1.1 kb	221	03169-L20603	
16p13.3	*TSC2*	Exon 26	1.4 kb	352	16736-L19347	«
16p13.3	*TSC2*	Exon 27	0.2 kb	371	01838-L20615	
16p13.3	*TSC2*	Exon 28	0.2 kb	136	16725-L19337	«
16p13.3	*TSC2*	Exon 29	0.7 kb	172	03170-L31381	«
16p13.3	*TSC2*	Exon 30	1.5 kb	395	01839-L31534	«
16p13.3	*TSC2*	Exon 31	0.8 kb	160	22266-L31542	«
16p13.3	*TSC2*	Exon 32	1.3 kb	274	11191-L31385	«
16p13.3	*TSC2*	Exon 33	0.9 kb	214	16730-L19341	«
16p13.3	*TSC2*	Exon 34	0.4 kb	409	22274-L31396	«
16p13.3	*TSC2*	Exon 35	0.3 kb	296	22262-L31543	«
16p13.3	*TSC2*	Exon 36	1.0 kb	428	22275-L31397	«
16p13.3	*TSC2*	Exon 37	0.5 kb	154	03171-L20600	«
16p13.3	*TSC2*	Exon 38	1.1 kb	453	01843-L30689	«
16p13.3	*TSC2*	Exon 39	0.2 kb	346	16723-L19335	«
16p13.3	*TSC2*	Exon 40	0.3 kb	202	16729-L19340	«
16p13.3	*TSC2*	Exon 41	0.1 kb	364	11935-L12755	«
16p13.3	*TSC2*	Exon 42	2.3 kb	460	22276-L31399	«
16p13.3	*PKD1*			310	22264-L27363	« ¬ ∫
2p	Reference			288	15880-L30312	
3p	Reference			500	09682-L22509	
5q	Reference			130	00797-L00463	
7p	Reference			244	16329-L30925	
11p	Reference			385	18677-L30318	
13q	Reference			466	05171-L04552	
15q	Reference			319	09767-L10182	
20p	Reference			418	21261-L29869	
22q	Reference			178	05458-L04861	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *TSC2* exon numbers are derived from MANE project and are based on MANE Select transcripts. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of several probes with targets at the edge of or slightly outside the coding region were altered. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

¬ This probe is a flanking probe, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probe are unlikely to be related to the condition tested, but could be an indication of *TSC2-PKD1* contiguous gene deletion syndrome. Solely copy number changes of *PKD1* are not expected to be the cause of Tuberos sclerosis complex.

« These probes are 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.

Ø These probes target sequences outside of the known coding region. Copy number alterations of only (one of) these probes are of unknown clinical significance.

∫ 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.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The use of the fixed cut-off values for the FR of the probes as mentioned in the table under section 5 will not allow the detection of deletions/duplications in all samples that are a mixture of normal and abnormal cells, such as mosaic samples (Blasco-Perez et al. 2023). Mosaicism is known to occur in ~5% (Kozlowski et al. 2007) up to 10-25% (Jang et al. 2012) of TSC patients. P046 TSC2 should be able to detect a deletion when it is present in at least 80% of the cells and a duplication when it is present in at least 40% of the cells. In order to detect mosaic samples, the analysis needs to have small 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).
2. This probemix is not suitable for the detection of *TSC2/PKD1* contiguous gene deletion syndrome. Although deletions involving *TSC2* may extend into the *PKD1* gene, the *PKD1* coverage in this probemix is insufficient to reliably detect deletions across *PKD1*. Suspected cases of *TSC2/PKD1* contiguous gene deletion syndrome should be further investigated using SALSA MLPA Probemix P351 PKD1 and SALSA MLPA Probemix P352 PKD1-PKD2.
3. No probes for *TSC2* exons 1 and 4 are present.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Jang MA et al. (2012). Identification of TSC1 and TSC2 mutations in Korean patients with tuberous sclerosis complex. *Pediatr Neurol*. 46:222-224.
2. Kozlowski P et al. (2007). Identification of 54 large deletions/duplications in TSC1 and TSC2 using MLPA, and genotype-phenotype correlations. *Hum Genet*. 121:389-400.
3. Mayer K et al. (2014). Clinical utility gene card for: tuberous sclerosis complex (TSC1, TSC2). *Eur J Hum Genet*. 22.
4. Blasco-Perez L et al. (2023). An Integral Approach to the Molecular Diagnosis of Tuberous Sclerosis Complex: The Role of Mosaicism and Splicing Variants. *J Mol Diagn*. 25:692-701.

Implemented changes in the product description

Version D1-04 – 02 March 2026 (03S)

- Product description updated to new template.
- Intended purpose updated to specify that assay is manual.
- The probemix-specific limitation regarding mosaicism has been updated.
- Inclusion of a limitation that the probemix is not suitable for the detection of *TSC2/PKD1* contiguous gene deletion syndrome.
- Inclusion of a limitation that the probemix does not cover *TSC2* exon 1 and 4.
- SNVs rs45517416 and rs766472320 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Warning for probes located in or near a GC-rich region (salt contamination) removed for probes: 01819-L20598, 03166-L31394, 01832-L01397, 21363-L30216, 16732-L30314, 22263-L20609, 01835-L20613, 16727-L19338, 03169-L20603 and 01838-L20615.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Warning for probes targeting sequences outside of the known coding region included for probes 16740-L31544 and 22270-L31391.
- Colombia added as country with IVD status.
- Probemix is now IVDR certified.