Instructions for Use

SALSA® MLPA® Probemix P337 TSC2 Confirmation

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 P337 TSC2 Confirmation product page on our website to find Certificates of Analysis and a list of related products.

Product name	**SALSA® MLPA® Probemix P337 TSC2 Confirmation**
Version	C1
Catalogue numbers	P337-025R (25 reactions) P337-050R (50 reactions) P337-100R (100 reactions)
Basic UDI-DI	872021148P3376A
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
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More information: www.mrcholland.com	
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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 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.

1. Intended Purpose

The SALSA MLPA Probemix P337 TSC2 Confirmation is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the confirmation of deletions or duplications in the *TSC2* gene as initially observed using the SALSA MLPA Probemix P046 TSC2, 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 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 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 P337 TSC2 Confirmation in 43 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 with exception of two samples that had one target probe falling slightly outside of the cut-off range. However, since the result of only one probe was ambiguous the correct genotype can be established in these and all other 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 P337 TSC2 Confirmation on two samples with known CNVs and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA. Only one minor deviation has been observed with one of the positive samples, with one target probe having a slightly higher FR than the cut-off with the use of 50 ng. This is acceptable, since only one probe is 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 P337 TSC2 Confirmation 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 70% of cells or a duplication in less than 50% of cells, P337 TSC2 Confirmation often produced false negatives. At 70% or higher for deletions, 50% or higher for duplications, P337 TSC2 Confirmation reliably detected the deletion/duplication, with most probes showing the expected FRs (≤0.8 for a deletion, ≥1.2 for a duplication).

<table>
<tr><td>Interfering substances</td><td>SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using P337 TSC2 Confirmation 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 (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 363/378 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 375/378 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 372/378 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 3/378 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 P337 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 P337 TSC2 Confirmation 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 1878/1890 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) (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*	Exon 1	0.8 kb	401	16739-L31799	+
16p13.3	*TSC2*	Exon 2	1.6 kb	172	11910-L12731	
16p13.3	*TSC2*	Exon 3	3.2 kb	357	13545-L20180	+
16p13.3	*TSC2*	Intron 4 (Exon 4)	0.7 kb	283	22271-L31392	Ø
16p13.3	*TSC2*	Exon 5	1.2 kb	199	14105-L31681	+
16p13.3	*TSC2*	Exon 6	0.7 kb	160	11908-L12729	
16p13.3	*TSC2*	Exon 7	0.6 kb	238	22300-L31421	+
16p13.3	*TSC2*	Exon 8	0.3 kb	256	11922-L12743	
16p13.3	*TSC2*	Exon 9	1.8 kb	337	22306-L31726	
16p13.3	*TSC2*	Exon 10	1.8 kb	296	11927-L19315	
16p13.3	*TSC2*	Exon 11	1.3 kb	193	11911-L31405	+
16p13.3	*TSC2*	Exon 12	0.5 kb	343	11932-L31426	
16p13.3	*TSC2*	Exon 13	0.7 kb	219	22299-L31415	+
16p13.3	*TSC2*	Exon 14	1.3 kb	373	11936-L30790	+
16p13.3	*TSC2*	Exon 15	1.3 kb	350	22303-L31684	
16p13.3	*TSC2*	Exon 16	4.7 kb	250	11921-L12742	+
16p13.3	*TSC2*	Exon 17	1.1 kb	309	22302-L31557	
16p13.3	*TSC2*	Exon 18	0.4 kb	427	22376-L31429	+
16p13.3	*TSC2*	Exon 19	0.3 kb	142	11905-L12726	«
16p13.3	*TSC2*	Exon 20	0.7 kb	384	11937-L30791	+
16p13.3	*TSC2*	Exon 21	1.3 kb	244	13543-L12735	
16p13.3	*TSC2*	Exon 22	1.5 kb	472	12803-L31153	«
16p13.3	*TSC2*	Exon 23	0.4 kb	453	13550-L31150	+
16p13.3	*TSC2*	Exon 24	0.4 kb	166	11909-L12730	«
16p13.3	*TSC2*	Exon 25	1.2 kb	462	11944-L31152	«
16p13.3	*TSC2*	Exon 26	1.3 kb	290	11926-L19314	«
16p13.3	*TSC2*	Exon 27	0.3 kb	301	11928-L19316	«
16p13.3	*TSC2*	Exon 28	0.3 kb	263	11923-L12744	«
16p13.3	*TSC2*	Exon 29	0.5 kb	154	22293-L30930	«
16p13.3	*TSC2*	Exon 30	1.7 kb	391	13546-L15221	« +
16p13.3	*TSC2*	Exon 31	0.7 kb	409	22307-L31427	«
16p13.3	*TSC2*	Exon 32	1.3 kb	226	22297-L31413	«
16p13.3	*TSC2*	Exon 33	0.4 kb	213	11915-L31409	«
16p13.3	*TSC2*	Exon 34	0.7 kb	490	16724-L31155	«
16p13.3	*TSC2*	Exon 35	0.3 kb	322	22261-L31683	« +
16p13.3	*TSC2*	Exon 36	1.0 kb	436	22305-L31424	« +
16p13.3	*TSC2*	Exon 37	0.5 kb	206	11913-L31408	«
16p13.3	*TSC2*	Exon 38	1.2 kb	136	11904-L18003	« +
16p13.3	*TSC2*	Exon 39	0.2 kb	269	22301-L31682	« +
16p13.3	*TSC2*	Exon 40	0.3 kb	481	12802-L31154	«
16p13.3	*TSC2*	Exon 41	0.2 kb	130	22292-L31556	«
16p13.3	*TSC2*	Exon 42	5.7 kb	418	21790-L31428	«
16p13.3	*PKD1*			315	22358-L24695	« ¬ ∫ +
1p	Reference			328	21544-L06206	
2p	Reference			500	19555-L27674	
4p	Reference			124	19616-L26275	
4q	Reference			148	04445-L03831	
5q	Reference			365	14059-L26885	
8q	Reference			178	10107-L10531	
9q	Reference			275	19811-L19312	
13q	Reference			445	21415-L08695	
17q	Reference			232	09641-L26943	

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. Description of a probe target at the edge of or slightly outside the coding region has been adjusted. No change in actual target site. 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 probes 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 TSC.

« 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 This probe targets sequences outside of the known coding region. Copy number alterations of only this probe are of unknown clinical significance.

+ The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequence sheet from the probemix-specific page on www.mrcholland.com.

∫ 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. P337 TSC2 Confirmation should be able to detect a deletion when it is present in at least 70% of the cells and a duplication when it is present in at least 50% 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. No probe for *TSC2* exon 4 is 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 C1-05 – 02 March 2026 (03S) - Product description updated to a new template. - Intended purpose updated to specify that assay is manual. - The probemix-specific limitation regarding mosaicism has been updated. - Included a limitation that *TSC2* exon 4 is not covered. - SNV(s) rs566118686 can affect the probe signal. However, the warning for this SNV present in previous product description versions has 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: 11910-L12731, 22376-L31429, 11937-L30791, 13543-L12735 and 13550-L31150. - 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 probe targeting a sequence outside of the known coding region included for probe 22271-L31392. - Warnings for ligations sites >20nt from the nearest exon were added for probes 16739-L31799, 13545-L20180, 14105-L31681, 22300-L31421, 22299-L31415, 11936-L30790, 11921-L12742, 11937-L30791, 13550-L31150, 13546-L15221, 22261-L31683, 22305-L31424, 11904-L18003, 11911-L31405, 22376-L31429, 22358-L24695 and 22301-L31682. - Probemix is now IVDR certified.