Product Description
SALSA® MLPA® Probemix P124-C3 TSC1

To be used with the MLPA General Protocol.

Version C3
As compared to version C2, one reference probe has been replaced. For complete product history see page 9.

Catalogue numbers:
- **P124-025R:** SALSA MLPA Probemix P124 TSC1, 25 reactions.
- **P124-050R:** SALSA MLPA Probemix P124 TSC1, 50 reactions.
- **P124-100R:** SALSA MLPA Probemix P124 TSC1, 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 P124 TSC1 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 *TSC1* gene in genomic DNA isolated from human peripheral whole blood specimens. P124 TSC1 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 P124 TSC1 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *TSC1* 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* gene.

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).

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

Gene structure
The *TSC1* gene spans ~54 kilobases (kb) on chromosome 9q34.13 and contains 23 exons. The *TSC1* LRG_486 is available at www.lrg-sequence.org and is identical to GenBank NG_012386.1.

Transcript variants
For *TSC1*, multiple variants have been described. Transcript variant 1 is the most predominant and encodes isoform 1 (NM_000368.5; 8598 nt; coding sequence 218-3712; http://www.ncbi.nlm.nih.gov/gene/7248). The ATG translation start site is located in exon 3 and the stop codon is located in exon 23.

Exon numbering
The *TSC1* exon numbering used in this P124-C3 TSC1 product description is the exon numbering from the LRG_486 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 P124-C3 TSC1 contains 32 MLPA probes with amplification products between 138 and 445 nucleotides (nt). This includes 23 probes for the *TSC1* gene. 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 NA13685 from the Coriell Institute has been tested with this P124-C3 probemix at MRC Holland and can be used as a positive control sample to detect a duplication of all TSC1 probes. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics
TSC1 deletions and duplications account for ~3% of all TSC cases (Mayer et al. 2014). The analytical sensitivity and specificity for the detection of deletions or duplications in the *TSC1* gene is very high and can be considered >99% (based on a 2005-2020 literature study performed by 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. 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.

P124 specific notes:

- A deletion of only exon 1 of *TSC1* gene, which is a non-coding exon, prevents *TSC1* expression and is thus a true null allele (van den Ouweland et al. 2011).
- 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 *TSC1* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P124 TSC1.
- 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 by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should 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.

Leiden Open Variation Database (LOVD) for *TSC1*

https://databases.lovd.nl/shared/genes/TSC1. We strongly encourage users to deposit positive results in the LOVD for the *TSC1* 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 *TSC1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P124-C3 TSC1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*TSC1*
64-105	Control fragments – see table in probemix content section for more information		
138	Reference probe 13335-L14761	18q21	
142	**TSC1 probe** 17487-L21739		**Exon 9**
148	**TSC1 probe** 04794-L21293		**Exon 11**
154	**TSC1 probe** 01846-L21291		**Exon 3**
172	**TSC1 probe** 04114-L03474		**Exon 13**
179	Reference probe 01963-L03341	20q13	
193	**TSC1 probe** 04117-L03477		**Exon 21**
202	**TSC1 probe** 17488-L21296		**Exon 23**
211	**TSC1 probe** 04112-L03472		**Exon 6**
228	**TSC1 probe** 04115-L21289		**Exon 17**
238	**TSC1 probe** 17489-L21297		**Exon 22**
250	**TSC1 probe** 04108-L21287		**Exon 1**
257	**TSC1 probe** 04797-L21288		**Exon 19**
264	Reference probe 17184-L21740	15q21	
279	**TSC1 probe** 09622-L21290		**Exon 2**
288	**TSC1 probe** 04795-L21294		**Exon 14**
300 ±	**TSC1 probe** 01849-L03718		**Exon 12**
310	Reference probe 06741-L06345	8q12	
316	**TSC1 probe** 15301-L03897		**Exon 18**
328	**TSC1 probe** 17490-L21298		**Exon 5**
339	Reference probe 06514-L20597	1q22	
346 *	Reference probe 17881-L22140	2p21	
364	**TSC1 probe** 04110-L21292		**Exon 4**
373	**TSC1 probe** 17486-L21286		**Exon 7**
382	**TSC1 probe** 01850-L13233		**Exon 15**
391 ±	Reference probe 01635-L01173	11q22	
403	**TSC1 probe** 17485-L21285		**Exon 20**
409	**TSC1 probe** 17491-L21299		**Exon 10**
418	**TSC1 probe** 04792-L04167		**Exon 8**
427	**TSC1 probe** 04796-L04171		**Exon 16**
433	Reference probe 06948-L06528	3q29	
445	Reference probe 03572-L03267	7q31	

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

* New in version C3 (from lot C3-1217 onwards).
± SNP rs118203504 influences the 300 nt probe signal (01849-L03718). SNP rs139646398 influences the 391 nt probe signal (01635-L01173). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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. *TSC1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*TSC1* exon[a]	Ligation site NM_000368.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
250	04108-L21287	Exon 1	44-45	GAGGGACTGTGA-GGTAAACAGCTG	9.5 kb
279	09622-L21290	Exon 2	133-134	GTTGTCGCTAGA-ACAGGTAAGCTA	6.2 kb
		start codon	*218-220 (Exon 3)*		
154	01846-L21291	Exon 3	255-256	GCTTCTTGCCAT-GCTGGACTCCCC	1.6 kb
364	04110-L21292	Exon 4	354-355	AAACACCTTGGT-GGATTATTACCT	1.6 kb
328	17490-L21298	Exon 5	519-520	AAGACTGCAGCC-ATCTTGGAAGCA	2.2 kb
211	04112-L03472	Exon 6	617-618	CAACAGGCGTCT-TGGTGTTGATAA	1.5 kb
373	17486-L21286	Exon 7	780-781	GTACGCACTCTT-TCATCGCCTTTA	0.5 kb
418	04792-L04167	Exon 8	906-907	TGTGCGAATTCA-TCCGGAATTAGT	9.0 kb
142	17487-L21739	Exon 9	1005-1006	TGCCAAAATCTC-TCTGGATCCCAC	0.9 kb
409	17491-L21299	Exon 10	1142-1143	GGTGTGCTACTT-CTACCCCTTACT	0.5 kb
148	04794-L21293	Exon 11	1298-1297 reverse	ATTTCCAGGAGA-AGTTGGAGGAGT	0.5 kb
300 ±	01849-L03718	Exon 12	1423-1424	CCACTCTGTCAT-TCGGATGACTAC	3.3 kb
172	04114-L03474	Exon 13	1508-1509	CTGCAAGACCAT-GTCTACACAGAC	0.5 kb
288	04795-L21294	Exon 14	1588-1589	GTCACTCTAAGT-GATCTTCCAGGG	0.9 kb
382	01850-L13233	Exon 15	1873-1874	AAGCAAGCCTTT-ACTCCCATAGAC	1.5 kb
427	04796-L04171	Exon 16	2235-2236	ACCCAGCAAGTC-TGTCGACTGGAC	0.6 kb
228	04115-L21289	Exon 17	2282-2283	CAGATGAGATCC-GCACCCTCCGAG	1.1 kb
316	15301-L03897	Exon 18	2527-2528	CAGCGTGACACT-ATGGTAACCAAG	1.0 kb
257	04797-L21288	Exon 19	2637-2638	CAGGAACATGAT-TGCGGAGCTGCG	0.9 kb
403	17485-L21285	Exon 20	2759-2760	AGATGGAGTTCT-TGAACAGGCAGC	3.3 kb
193	04117-L03477	Exon 21	2916-2917	CCAGCAGACTCA-GAGGCTTGATAC	0.3 kb
238	17489-L21297	Exon 22	3132-3133	GTTGGAGAAAGA-TGGCCTCCTGAA	0.5 kb
202	17488-L21296	Exon 23	3233-3234	ATTCCATGGTAG-GGCACAATGAAG	
		stop codon	*3710-3712 (Exon 23)*		

[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.

± SNP rs118203504 influences the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.
- P337 TSC2 Confirmation: P337 (version C1 or higher) can be used for confirmation of results obtained from P046 TSC2 (version D1 or higher). 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.
- P351/P352 PKD1-PKD2: These probemixes contain probes for the *PKD1* and *PKD2* genes.

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P124 product history	
Version	*Modification*
C3	One reference probe has been replaced.
C2	The length of one probe has been adjusted.
C1	Five probes for *TSC1* and six reference probes have been replaced, and three reference probes have been removed. QDX2 fragments have been added.
B1	The probes for *TSC1* exons 2, 9 and 22 have been replaced. In addition, extra control fragments have been added.
A1	First release.

Implemented changes in the product description
Version C3-03 – 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 NA13685 added. - Added note on how to interpret a deletion of only exon 1 of the *TSC1* gene and information about mosaicism in the interpretation of results section. - Israel added as country with IVD status. - UK has been added to the list of countries in Europe that accept the CE mark. - Ligation sites of the probes targeting the *TSC1* gene updated according to new version of the NM_ reference sequence. - Warnings added to Table 1 and 2 for the effect of SNPs rs118203504 and rs139646398 on the probe ratio of probes 01849-L03718 and 01635-L01173, respectively. - New references added. Version C3-02 – 09 November 2018 (04) - Chromosomal location of TSC1 corrected in the Gene structure section. Version C3-01 – 16 February 2018 (04) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). Version 13 – 23 February 2016 (55) - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). - Small change in the length of probe 13335-L14761 in Table 1 in order to better reflect the true length of the amplification product.

More information: www.mrcholland.com; www.mrcholland.eu	
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IVD	EUROPE* CE ISRAEL
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*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.