Product Description SALSA® MLPA® Probemix P124-C3 TSC1

To be used with the MLPA General Protocol.

P124 Version C3. As compared to version C2, one reference probe has been replaced. For complete product history see page 7.

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, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 use: The SALSA MLPA probemix P124 TSC1 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *TSC1* gene, in order to confirm a potential cause and clinical diagnosis for tuberous sclerosis complex (TSC). This product can also be used for molecular genetic testing of at-risk family members. This assay is for use with human DNA extracted from peripheral blood and not for use with genomic DNA extracted from formalin-fixed paraffin embedded or fresh tumour material.

Deletions or duplications detected with the P124 TSC1 probemix must be confirmed by using another technique. In particular, deletions or duplications detected by only a single probe always require validation 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 SALSA MLPA probemix in combination with sequence analysis of the *TSC1* gene. This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Tuberous sclerosis complex (TSC) is a genetic disorder characterized 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. 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 (51%–82%) of all TSC patients as compared to *TSC1* mutations (~24%) (Sancak et al. 2005). *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 (6%) (Mayer et al. 2014). 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).

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

Gene structure: The *TSC1* gene spans ~54 kb of genomic DNA and is located on chromosome 9q34.13. The *TSC1* LRG_486 is available at http://www.lrg-sequence.org and is identical to GenBank NG_012386.1.

Transcript variants: *TSC1* LRG_486 is identical to transcript NM_000368.4 and is described on https://www.ncbi.nlm.nih.gov/gene/7248. This sequence is a reference standard in the RefSeqGene project. The ATG translocation start site is located in exon 3 (235-237) and the stop codon is located in exon 23 (3727-3729).

Exon numbering: The exon numbering used in this P124-C3 TSC1 product description is the exon numbering from the RefSeq transcript NM_000368.4, which is identical to the LRG_486 sequence. The exon numbering and NM sequence used are from January 2018, but can be changed (e.g. by NCBI) after the release of the product description.

Probemix content: The P124-C3 probemix contains 32 probes with amplification products between 138 and 445 nt. This probemix contains one probe for each exon of the *TSC1* gene. In addition, 9 reference probes are included in the P124-C3 *TSC1* probemix, detecting several different autosomal chromosomal locations. The identity of the genes detected by the reference probes is available online (www.mlpa.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, 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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)
88-96	D-fragments (Low signal of 88 or 96 fragment 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.mlpa.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 peripheral blood, 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: 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 unrelated individuals who are from families without a history of TSC. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a

positive control DNA sample in your MLPA experiments. 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 2005-2016 literature study).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) 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 must 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.mlpa.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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases (van Veghel-Plandsoen et al. 2011). 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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 (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 in some cases not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Limitations of the procedure:

- In most populations, the major causes of genetic defects in the *TSC1* gene are small (point) mutations, most of which will not 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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 one or more than one consecutive probe (Table 2) 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.

Tuberous sclerosis mutation database:
http://chromium.lovd.nl/LOVD2/TSC/home.php?select_db=TSC1. We strongly encourage users to deposit positive results in the tuberous sclerosis mutation database. 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 SNPs and unusual results (e.g., a duplication of *TSC1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.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) The exon numbering used in this P124 TSC1 product description is the exon numbering from the RefSeq transcript NM_000368.4, which is identical to the LRG_486 sequence. Exon numbering used here may differ from literature. Please notify us of any mistakes.

* New in version C3 (from lot C3-1217 onwards).

Table 2. *TSC1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*TSC1* Exon[a]	Ligation site NM_000368.4[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*235-237 (exon 3)*		
250	04108-L21287	Exon 1	61-62	GAGGGACTGTGA-GGTAAACAGCTG	9.5 kb
279	09622-L21290	Exon 2	150-151	GTTGTCGCTAGA-ACAGGTAAGCTA	6.2 kb
154	01846-L21291	Exon 3	272-273	GCTTCTTGCCAT-GCTGGACTCCCC	1.6 kb
364	04110-L21292	Exon 4	371-372	AAACACCTTGGT-GGATTATTACCT	1.6 kb
328	17490-L21298	Exon 5	536-537	AAGACTGCAGCC-ATCTTGGAAGCA	2.2 kb
211	04112-L03472	Exon 6	634-635	CAACAGGCGTCT-TGGTGTTGATAA	1.5 kb
373	17486-L21286	Exon 7	797-798	GTACGCACTCTT-TCATCGCCTTTA	0.5 kb
418	04792-L04167	Exon 8	923-924	TGTGCGAATTCA-TCCGGAATTAGT	9.0 kb
142	17487-L21739	Exon 9	1022-1023	TGCCAAAATCTC-TCTGGATCCCAC	0.9 kb
409	17491-L21299	Exon 10	1159-1160	GGTGTGCTACTT-CTACCCCTTACT	0.5 kb
148	04794-L21293	Exon 11	1315-1314 *reverse*	ATTTCCAGGAGA-AGTTGGAGGAGT	0.5 kb
300	01849-L03718	Exon 12	1440-1441	CCACTCTGTCAT-TCGGATGACTAC	3.3 kb
172	04114-L03474	Exon 13	1525-1526	CTGCAAGACCAT-GTCTACACAGAC	0.5 kb
288	04795-L21294	Exon 14	1605-1606	GTCACTCTAAGT-GATCTTCCAGGG	0.9 kb
382	01850-L13233	Exon 15	1890-1891	AAGCAAGCCTTT-ACTCCCATAGAC	1.5 kb
427	04796-L04171	Exon 16	2252-2253	ACCCAGCAAGTC-TGTCGACTGGAC	0.6 kb
228	04115-L21289	Exon 17	2299-2300	CAGATGAGATCC-GCACCCTCCGAG	1.1 kb
316	15301-L03897	Exon 18	2544-2545	CAGCGTGACACT-ATGGTAACCAAG	1.0 kb
257	04797-L21288	Exon 19	2654-2655	CAGGAACATGAT-TGCGGAGCTGCG	0.9 kb
403	17485-L21285	Exon 20	2776-2777	AGATGGAGTTCT-TGAACAGGCAGC	3.3 kb
193	04117-L03477	Exon 21	2933-2934	CCAGCAGACTCA-GAGGCTTGATAC	0.3 kb
238	17489-L21297	Exon 22	3149-3150	GTTGGAGAAAGA-TGGCCTCCTGAA	0.5 kb
202	17488-L21296	Exon 23	3250-3251	ATTCCATGGTAG-GGCACAATGAAG	
		stop codon	*3727-3729 (exon 23)*		

(a) The exon numbering used in this P124 TSC1 product description is the exon numbering from the RefSeq transcript NM_000368.4, which is identical to the LRG_486 sequence.
(b) Ligation sites of the P124-C3 TSC1 MLPA probes are indicated according to RefSeq sequence NM_000368.4 containing 23 exons.
(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA® MLPA® probemixes

- P046 TSC2: This probemix contains probes for the *TSC2* gene.
- P337 TSC2: Contains more probes for the *TSC2* gene. This probemix can be used for confirmation of obtained P046 results.
- P351 P352 PKD1 PKD2: These probemixes contain probes for the *PKD1* and *PKD2* genes.

References

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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 C3-02 – 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. *Version 12 – 23 July 2015 (54)* - Figure based on the use of old MLPA buffer (replaced in December 2012) removed. - Various minor textual changes throughout the document. *Version 11 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

More information: www.mlpa.com; www.mlpa.eu	
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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

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