Instructions for Use
SALSA® MLPA® Probemix P124 TSC1

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 P124 TSC1 product page on our website to find Certificates of Analysis and a list of related products.

Product name	**SALSA® MLPA® Probemix P124 TSC1**
Version	C3
Catalogue numbers	P124-025R (25 reactions) P124-050R (50 reactions) P124-100R (100 reactions)
Basic UDI-DI	872021148P1245P
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 ISRAEL
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 C2, one reference probe has been replaced.

1. Intended Purpose

The SALSA MLPA Probemix P124 TSC1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions 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 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, 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 (*TSC1*)

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 P124 TSC1 in 42 samples from healthy individuals with normal copy number and three control plasmid 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 recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P124 TSC1 on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA. Internal testing was carried out on P124 TSC1 to investigate the effect of mosaicism on probe performance. Mosaicism was simulated by mixing two DNA samples carrying heterozygous *TSC1* deletions with varying amounts of normal DNA, creating mixtures with 40-90% of deletion 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 60-70% of cells, P124 TSC1 often produced false negatives. At 60-70% or higher, P124 TSC1 reliably detected the deletion, with most probes showing the expected reduced FRs (≤0.80).
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 P124 TSC1 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on three control plasmid samples with known CNVs and one sample without any mutation. 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.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 274/276 measurements

	NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 275/276 measurements
	Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 275/276 measurements
	Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 276/276 measurements
	Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 268/276 measurements

* Results are summarised for all probes across all four samples tested.

Heparin did not interfere with copy number determination, while an effect on the final ratios (FRs) was observed for a low number of probes with EDTA, NaCl and Fe^{3+}. Hemoglobin had the largest effect on the FRs. DNA extraction methods from blood remove hemoglobin. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found. Coffalyser.Net issues warnings for the samples in which hemoglobin showed an effect, as well as lowered quality scores, leading to the samples needing a re-test.

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.

Cross-reactivity	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.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, a previously genotyped sample was tested using P124 TSC1 and found to have the expected result. Assay precision was tested by repeatedly testing control plasmid samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 1034/1035 data points, leading to a precision of >99%.
Clinical validity*	*TSC1:* Around 3% of TSC cases are caused by CNVs in *TSC1* (Mayer et al. 2014). *(Based on a 2000-2025 literature review)

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number
9q34.13	*TSC1*	Exon 23	0.5 kb	202	17488-L21296
9q34.13	*TSC1*	Exon 22	0.3 kb	238	17489-L21297
9q34.13	*TSC1*	Exon 21	3.3 kb	193	04117-L03477
9q34.13	*TSC1*	Exon 20	0.9 kb	403	17485-L21285
9q34.13	*TSC1*	Exon 19	1.0 kb	257	04797-L21288
9q34.13	*TSC1*	Exon 18	1.1 kb	316	15301-L03897
9q34.13	*TSC1*	Exon 17	0.6 kb	228	04115-L21289
9q34.13	*TSC1*	Exon 16	1.5 kb	427	04796-L04171
9q34.13	*TSC1*	Exon 15	0.9 kb	382	01850-L13233
9q34.13	*TSC1*	Exon 14	0.5 kb	288	04795-L21294
9q34.13	*TSC1*	Exon 13	3.3 kb	172	04114-L03474
9q34.13	*TSC1*	Exon 12	0.5 kb	300	01849-L03718
9q34.13	*TSC1*	Exon 11	0.5 kb	148	04794-L21293
9q34.13	*TSC1*	Exon 10	0.9 kb	409	17491-L21299
9q34.13	*TSC1*	Exon 9	9.0 kb	144	17487-L21739
9q34.13	*TSC1*	Exon 8	0.5 kb	418	04792-L04167
9q34.13	*TSC1*	Exon 7	1.5 kb	373	17486-L21286
9q34.13	*TSC1*	Exon 6	2.2 kb	211	04112-L03472
9q34.13	*TSC1*	Exon 5	1.6 kb	328	17490-L21298
9q34.13	*TSC1*	Exon 4	1.6 kb	365	04110-L21292
9q34.13	*TSC1*	Exon 3	6.2 kb	154	01846-L21291
9q34.13	*TSC1*	Exon 2	9.5 kb	279	09622-L21290
9q34.13	*TSC1*	Exon 1		250	04108-L21287
1q	Reference			339	06514-L20597
2p	Reference			346	17881-L22140
3q	Reference			433	06948-L06528
7q	Reference			445	03572-L03267
8q	Reference			310	06741-L06345
11q	Reference			391	01635-L01173
15q	Reference			265	17184-L21740
18q	Reference			138	13335-L14761
20q	Reference			179	01963-L03341

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 *TSC1* 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.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings
There are no probe warnings.

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. 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).
2. 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. Mosaicism is known to occur in ~5% (Kozlowski et al. 2007) up to 10-25% (Jang et al. 2012) of TSC patients. P124 TSC1 should be able to detect a deletion when it is present in at least 60-70% 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).

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. van den Ouweland AM et al. (2011). Characterisation of TSC1 promoter deletions in tuberous sclerosis complex patients. *Eur J Hum Genet.* 19:157-163.

Implemented changes in the product description
Version C3-04 – 03 February 2026 (03S) - Product description adapted to a new template. - Intended purpose updated to specify that assay is manual and removal of the detection of duplications in *TSC1* due to lack of clinical evidence. - The probemix-specific limitation regarding mosaicism has been updated. - SNV(s) rs118203504 and rs139646398 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. - Probemix-specific limitation adjusted for mosaic samples. - Probemix is now IVDR certified.