Product Description SALSA® MLPA® probemix P101-B4 STK11

To be used with the MLPA General Protocol.

Version B4. Compared to version B3, one reference probe has been replaced and the LDLR probe has been replaced by a reference probe. For complete product history see page 8.

Catalogue numbers:
- **P101-025R:** SALSA® MLPA® probemix P101 STK11, 25 reactions.
- **P101-050R:** SALSA® MLPA® probemix P101 STK11, 50 reactions.
- **P101-100R:** SALSA® MLPA® probemix P101 STK11, 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.

Please note that the complete *STK11* gene region, and in particular the exon 9-10 region, is extremely GC-rich and is therefore difficult to denature. The use of DNA samples containing 20 mM or more salt can result in false positive deletion results. In particular, the use of Qiagen EZ1, M48 and M96 systems frequently results in DNA denaturation problems as these systems can result in very high salt concentrations. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration). When using silica column based DNA purification, salt concentrations can often be reduced by inclusion of a wash step with 85% ethanol before the elution step.

A low signal of the 88 nt and 96 nt DNA denaturation control fragments provides a warning for incomplete DNA denaturation. Two probes (159 nt & 178 nt) located in GC-rich regions have been included in this P101-B4 probemix. However, these probes are less sensitive to salt contamination than the *STK11* probes. **Please note** that at certain salt concentrations, both the *STK11* exon 9 and exon 10 probes may show reduced probe signals even in the absence of a denaturation warning in the Coffalyser.Net software.

Intended use: The SALSA MLPA probemix P101 STK11 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *STK11* gene, in order to confirm a potential cause and clinical diagnosis for Peutz-Jeghers syndrome. This assay is for use with human DNA extracted from peripheral blood. This product can also be used for molecular genetic testing of at-risk family members.

Deletions or duplications obtained with the P101 STK11 probemix must be confirmed by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in the *STK11* gene are small indel-type 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 *STK11* gene. This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test must 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 the product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Peutz-Jeghers syndrome (PJS) is an autosomal dominant disorder characterized by benign gastrointestinal polyps, hyper-pigmented skin spots, and an increased risk (>15x) of malignant

epithelial cancers at various anatomic sites (colorectal, gastric, pancreatic, breast, uterine cervix, and ovarian cancers). The prevalence of this condition is uncertain; estimates range from 1 in 25.000 to 300.000 individuals. The basis of familial PJS is a germline mutation in the *STK11* tumour suppressor gene, located in chromosomal region 19p13.3.

STK11 alterations in PJS patients comprise mainly point mutations and it is estimated that ~15% of pathogenic mutations in the *STK11* gene are attributed to large deletions/duplications, which is comparable between PJS populations (Borun et al. 2015, Chow et al. 2006, Orellana et al. 2013). The *STK11* gene is frequently inactivated by deletions or by point mutations in several cancer types, including lung and cervical cancer, and inactivation is suggested to be associated with disease progression (Ji et al. 2007, Wingo et al. 2009).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1266/.

Gene structure and transcript variant: The *STK11* gene spans ~23 kb of genomic DNA on chromosome 19p13.3 and contains 10 exons. The *STK11* LRG_319 is identical to RefSeq transcript NM_000455.4 (www.lrg-sequence.org/). Transcript variant NM_000455.4 (3286 bp, coding sequence 1116-2417) is described here: https://www.ncbi.nlm.nih.gov/gene/6794.

Exon numbering: The exon numbering used in this P101-B4 STK11 product description is the exon numbering from the RefSeq transcript NM_000455.4, which is identical to the LRG_319 sequence. The exon numbering and NM sequence used is from February 2018, but can be changed (e.g. by NCBI) after the release of the product description.

P101-B4 probemix content: This SALSA MLPA P101 STK11 probemix contains 27 probes with amplification products between 150 and 391 nt: 12 probes for the *STK11* gene (one probe for each exon and three probes for exon 1), 3 probes for genes located upstream of *STK11* (*CDC34*, *ELANE* and *KISS1R*), 2 DNA denaturation probes, and 10 reference probes detecting sequences on other chromosomes. 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 121 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.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 Peutz-Jeghers syndrome. 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: The expected number of *STK11* deletions that can be detected with this MLPA probemix is ~15% of all mutations in patients with Peutz-Jeghers syndrome (Borun et al. 2015). The analytical sensitivity and specificity for the detection of deletions in *STK11* is very high and can be considered >99% (based on a 2005-2016 literature review).

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. 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 exon 9 and 10 of the *STK11* gene. The complete *STK11* gene is located in a very 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 cause of genetic defects in the *STK11* gene are small indel-type mutations, none of which will be detected by using SALSA® MLPA® probemix P101 STK11.
- 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.
- The diagnostic use of P101 with DNA extracted from tumour tissue has not been validated.
- 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 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.

***STK11* mutation database:** http://databases.lovd.nl/shared/genes/STK11. We strongly encourage users to deposit positive results in the *STK11* gene 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 *STK11* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA® MLPA® probemix P101-B4 STK11

Length (nt)	SALSA MLPA probe	Chromosomal position[a] reference	STK11
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)		
88-92-96	D-fragments (Low signal of 88 or 96 fragment indicates incomplete denaturation)		
100	X-fragment (X chromosome specific)		
105	Y-fragment (Y chromosome specific)		
150	Reference probe 01171-L00616	8q22	
159 «	DNA denaturation probe 05523-L04951	20q13	
169 « #	**STK11 probe** 03891-L03986		**Exon 10**
178 «	DNA denaturation probe 14170-L17280	22q13	
184	Reference probe 13261-L14594	1p21	
195 «	**STK11 probe** 03124-L03988		**Exon 1**
202 « ¬	Flanking probe 3 11955-L18080		19p13.3; 285 kb telomeric
214 ¬	Flanking probe 2 07916-L07646		19p13.3; 350 kb telomeric
221 «	**STK11 probe** 03125-L18081		**Exon 2**
229 «	**STK11 probe** 03126-L18082		**Exon 3**
238 «	**STK11 probe** 03127-L03338		**Exon 4**
247 «	**STK11 probe** 02215-L10041		**Exon 1**
256	Reference probe 02336-L01821	12q23	
265 « ¬	Flanking probe 1 01737-L01313		19p13.3; 664 kb telomeric
274	Reference probe 02470-L01914	15q21	
283 «	**STK11 probe** 16639-L19597		**Exon 5**
292 «	**STK11 probe** 03129-L03340		**Exon 6**
301 *	Reference probe 07636-L07321	19p13.2; 10 Mb centromeric	
310	Reference probe 15380-L17211	3p22	
317 «	**STK11 probe** 16638-L19168		**Exon 7**
328	Reference probe 02663-L02130	11q22	
337 «	**STK11 probe** 03131-L02583		**Exon 8**
346 « #	**STK11 probe** 03132-L03990		**Exon 9**
355	Reference probe 15081-L16844	4q31	
373 « ±	**STK11 probe** 02251-L10042		**Exon 1**
382 *	Reference probe 20537-L28127	1q31	
391	Reference probe 17885-L22144	2p21	

(a) The exon numbering used in this P101-B4 STK11 product description is the exon numbering from the RefSeq transcript NM_000455.4, which is identical to the LRG_319 sequence.

* New in version B4 (from lot B4-0317 onwards).
± SNP rs372994361 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« Probe 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, especially of GC-rich regions.
In case of apparent (partial) deletions please check if the 88 nt and 96 nt control fragments also show a reduced ratio. If this is the case, the sample probably contains a high salt concentration as these probes are very salt-sensitive. Please note that at certain salt concentrations, both the *STK11* exon 9 and exon 10 probes may show reduced probe signals even in the absence of a denaturation warning in the Coffalyser.Net software.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Table 2. STK11 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site[b] NM_000455.4	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
					492 kb from 19p telomere to flanking probe 1
265 « ¬	01737-L01313	Flanking probe 1		CTCTTCTACGAC-GACTACTACGAG	314.6 kb
214 ¬	07916-L07646	Flanking probe 2		GATCGACTCTAT-CATCCAACGCTC	64.4 kb
202 « ¬	11955-L18080	Flanking probe 3		GTCCTACAGCAA-CTCCGCGCTGAA	285.3 kb
STK11 probes					
		start codon	*1116-1118 (exon 1)*		
247 «	02215-L10041	Exon 1	3-4	ATGGCGGCGGCG-TGTCGGGCGCGG	0.8 kb
373 « ±	02251-L10042	Exon 1	840-841	TGAGGCCCGGGT-CCCACTGGAACT	0.3 kb
195 «	03124-L03988	Exon 1	1183-1184	GGTGGGTATGGA-CACGTTCATCCA	11.5 kb
221 «	03125-L18081	Exon 2	1439-1440	TTACGGCACAAA-AATGTCATCCAG	0.9 kb
229 «	03126-L18082	Exon 3	1530-1531	GCATGCAGGAAA-TGCTGGACAGCG	1.1 kb
238 «	03127-L03338	Exon 4	1619-1620	GAGTACCTGCAT-AGCCAGGGCATT	0.2 kb
283 «	16639-L19597	Exon 5	1740-1741	CGGCGGACGACA-CCTGCCGGACCA	0.7 kb
292 «	03129-L03340	Exon 6	1906-1907	CTACAAGTTGTT-TGAGAACATCGG	0.7 kb
317 «	16638-L19168	Exon 7	1994-1995	CTTGAGTACGAA-CCGGCCAAGAGG	1.2 kb
337 «	03131-L02583	Exon 8	2189-2188 reverse	TAGATGATGTCA-TCCTCGATGTCG	3.4 kb
346 « #	03132-L03990	Exon 9	2289-2290	AGGCCGTGTGTA-TGAACGGCACAG	1.1 kb
169 « #	03891-L03986	Exon 10	2480-2481	GGCCCTCAGTCT-TCCTGCCGGTTC	
		stop codon	*2415-2417 (exon 9)*		

(a) The exon numbering used in this P101-B4 STK11 product description is the exon numbering from the RefSeq transcript NM_000455.4, which is identical to the LRG_319 sequence.
(b) Ligation sites of the P101 STK11 MLPA probes are indicated according to RefSeq sequence NM_000455.4 containing 10 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.

± SNP rs372994361 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« Probe 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, especially of GC-rich regions.
In case of apparent (partial) deletions please check if the 88 nt and 96 nt control fragments also show a reduced ratio. If this is the case, the sample probably contains a high salt concentration as these probes are very salt-sensitive. Please note that at certain salt concentrations, both the *STK11* exon 9 and exon 10 probes may show reduced probe signals even in the absence of a denaturation warning in the Coffalyser.Net software.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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P101 Product history	
Version	*Modification*
B4	One reference probe has been replaced and the LDLR probe has been replaced by a reference probe.
B3	One reference probe has been replaced and one flanking probe has been replaced by a reference probe.
B2	One reference probe has been replaced and one has been added.
B1	Two STK11 probes, 3 reference probes and 2 control fragments (88 and 96 nt) have been replaced. One STK11 exon 1 probe has been removed.
A2	One reference probe has been replaced and two STK11 probes have a small change in length / peak height. Extra control fragments at 100 and 105 nt have been added.
A1	First release.

Implemented changes in the product description

Version B4-04 – 18 December 2018 (04)
- Regulatory status section updated.
- Selected publications using P101 STK11 updated.

Version B4-03 – 09 February 2018 (04)
- Product description adapted to a new template.
- An extra warning with explanation for the salt sensitive STK11 probes, in particular those in exon 9 and exon 10, was added to the section Precautions and warnings and to Table 1 and 2.
- The minimum salt concentration leading to DNA denaturation problems for STK11 has been updated from 40 mM salt to 20 mM salt due to newly obtained information with the P101 probemix.

Version B4-02 – 13 June 2017 (03)
- The size of the transcript variant has been changed on page 2.

Version B4-01 – 29 May 2017 (03)
- 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 21 – 14 January 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 20 – 04 June 2015 (54)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Minor textual changes.

Version 19 (53)
- Minor textual and lay-out changes.

Version 18 (53)
- Product description adapted to a new version (lot number added, small changes in Table 1 and Table 2, new picture included).

More information: www.mlpa.com; www.mlpa.eu	
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