Product Description SALSA® MLPA® Probemix P333-A4 EP300

To be used with the MLPA General Protocol.

Version A4. As compared to version A3 one reference probe has been removed and two reference probes have been replaced. For complete product history see page 6.

Catalogue numbers:
- **P333-025R:** SALSA MLPA Probemix P333 EP300, 25 reactions.
- **P333-050R:** SALSA MLPA Probemix P333 EP300, 50 reactions.
- **P333-100R:** SALSA MLPA Probemix P333 EP300, 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.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.

General information: The SALSA MLPA Probemix P333 EP300 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *EP300* gene, which is associated with Rubinstein-Taybi syndrome (RSTS).

RSTS is characterised by growth retardation, distinctive facial dysmorphisms, skeletal abnormalities such as broad and often angulated thumbs, and intellectual disability. RSTS is caused by mutations in two genes: *EP300*, encoding E1A-associated protein p300, and *CREBBP*, encoding a CREB-binding protein. Approximately, 3-8% of RSTS individuals carry heterozygous mutations in *EP300*, while pathogenic variants in *CREBBP* are identified in approximately 50-60% of RSTS patients.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1526/.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering: The *EP300* and *CREBBP* exon numbering used in this P333-A4 EP300 product description is the exon numbering from the RefSeq transcripts NM_001429.4 and NM_004380.3, which are identical to the LRG_1422 and LRG_1426 sequences, respectively. The exon numbering and NM_ sequence used have been retrieved on 03/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P333-A4 EP300 contains 41 MLPA probes with amplification products between 136 and 463 nucleotides (nt). This includes 31 probes for the *EP300* gene, one probe for each exon of the gene, and one probe for the *CREBBP* 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.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, 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 nt and 96 nt 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 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 unrelated individuals who are from families without a history of RSTS. 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 Institute (https://catalog.coriell.org) and Leibniz Institute 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.

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.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 standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *EP300* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P333 EP300.
- 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 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.

***EP300* mutation database:** https://databases.lovd.nl/shared/genes/EP300. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). 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., duplication of *EP300* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P333-A4 EP300

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	EP300	CREBBP
64-105	Control fragments – see table in probemix content section for more information			
136	Reference probe 17217-L20544	8q21		
142	**EP300 probe** 09926-L10385		**Exon 2**	
148	Reference probe 13226-L14559	1p21		
154	**EP300 probe** 09934-L10393		**Exon 28**	
160	**EP300 probe** 12263-L13206		**Exon 19**	
166	**EP300 probe** 12264-L13207		**Exon 27**	
172 *	Reference probe 10922-L11591	9q34		
178	**EP300 probe** 09933-L10392		**Exon 24**	
184	**EP300 probe** 12265-L13208		**Exon 7**	
190	**EP300 probe** 12266-L13209		**Exon 25**	
201 «	**EP300 probe** 09925-L11195		**Exon 1**	
208	**EP300 probe** 12267-L29495		**Exon 15**	
214	**EP300 probe** 12268-L13211		**Exon 20**	
226	**EP300 probe** 12269-L13212		**Exon 17**	
232	**EP300 probe** 20991-L29494		**Exon 3**	
238	**EP300 probe** 09931-L10390		**Exon 18**	
250	**EP300 probe** 12271-L13214		**Exon 30**	
256	**EP300 probe** 12272-L13215		**Exon 23**	
264	**EP300 probe** 13429-L15124		**Exon 31**	
274	**EP300 probe** 13427-L14882		**Exon 8**	
285	**EP300 probe** 09932-L11740		**Exon 21**	
293	**EP300 probe** 12273-L13216		**Exon 11**	
301	**EP300 probe** 12274-L13217		**Exon 6**	
310	Reference probe 10463-L11016	11q13		
319	**EP300 probe** 12275-L13218		**Exon 16**	
328	Reference probe 11652-L12416	5q33		
337	**EP300 probe** 12276-L13219		**Exon 26**	
346	**CREBBP probe** 09896-L10309			**Exon 17**
355	**EP300 probe** 09930-L10389		**Exon 12**	
364	**EP300 probe** 12277-L13220		**Exon 9**	
373	**EP300 probe** 12278-L13221		**Exon 22**	
381 *	Reference probe 14422-L24469	12q21		
391	**EP300 probe** 09929-L10388		**Exon 10**	
400	**EP300 probe** 12279-L13222		**Exon 5**	
409	**EP300 probe** 12280-L13223		**Exon 14**	
418	Reference probe 10612-L11163	15q15		
425	**EP300 probe** 12281-L13224		**Exon 29**	
436	**EP300 probe** 12282-L13225		**Exon 13**	
445	**EP300 probe** 09927-L10386		**Exon 4**	
454	Reference probe 10717-L11299	6p12		
463	Reference probe 17892-L27981	19q13		

a) See above section on exon numbering for more information.

* New in version A4.
« 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.

Table 2. P333-A4 probes arranged according to chromosomal location

Table 2a. *EP300*

Length (nt)	SALSA MLPA probe	EP300 exon[a]	Ligation site NM_001429.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*414-416 (Exon 1)*		
201 «	09925-L11195	Exon 1	412-413	GAAAGAATTAAA-AATGGCCGAGAA	24.6 kb
142	09926-L10385	Exon 2	871-872	GTCAACAGGTAT-GATGAACAGTCC	8.4 kb
232	20991-L29494	Exon 3	1244-1245	ATTCAGACAAAA-ACTGTACTATCA	1.7 kb
445	09927-L10386	Exon 4	1541-1542	AAGAATGTCCTA-AACCACATGACA	2.2 kb
400	12279-L13222	Exon 5	1616-1617	CAAATCATTTCA-CACTGGAAGAAT	1.6 kb
301	12274-L13217	Exon 6	1843-1844	ACCCTATCAAGT-AAATCAGATGCC	4.3 kb
184	12265-L13208	Exon 7	1962-1963	CTATGGGAGTAA-ATGGAGGTGTAG	1.8 kb
274	13427-L14882	Exon 8	2053-2054	GATGAGTGAAAA-TGCCAGTGTGCC	2.5 kb
364	12277-L13220	Exon 9	2220-2221	CTTTAAAAGACA-GACGGATGGAAA	0.9 kb
391	09929-L10388	Exon 10	2315-2316	CACCTTCTAGCT-GAGAAAATCTAT	5.7 kb
293	12273-L13216	Exon 11	2489-2490	CCTGACCCAAGT-ATGATCCGTGGC	1.2 kb
355	09930-L10389	Exon 12	2628-2629	ACCATGGACAGT-TGGCTCAACCTG	1.1 kb
436	12282-L13225	Exon 13	2680-2681	GCCTCGTATGCA-ACAGCCTTCCAA	1.0 kb
409	12280-L13223	Exon 14	3085-3086	ACCACCAACAAC-ACAACTTCCCCA	1.8 kb
208	12267-L29495	Exon 15	3275-3276	GTATCAAATCCT-CCATCTACTAGT	0.4 kb
319	12275-L13218	Exon 16	3501-3502	AGGAAGACCAGC-CAAGTACTTCAG	2.8 kb
226	12269-L13212	Exon 17	3625-3626	TCAGGATCCAGA-ATCCCTTCCCTT	2.2 kb
238	09931-L10390	Exon 18	3723-3724	CTACCATTAAGA-GGAAGTTAGACA	1.3 kb
160	12263-L13206	Exon 19	3981-3982	CTCGTGATGCCA-CTTATTACAGTT	2.2 kb
214	12268-L13211	Exon 20	4053-4054	GGGAGAGCGTTT-CTTTGGGGGATG	2.1 kb
285	09932-L11740	Exon 21	4141-intron 21	GGATCCTGAACT-GTAAGTACGATC	1.3 kb
373	12278-L13221	Exon 22	4157-4158	GTTGAATGTACA-GAGTGCGGAAGA	2.6 kb
256	12272-L13215	Exon 23	4248-4249	GTTTAAAGAAAA-GTGCACGAACTA	1.9 kb
178	09933-L10392	Exon 24	4365-4366	ATCACCCTGAGT-CAGGAGAGGTCA	0.2 kb
190	12266-L13209	Exon 25	4463-4464	GGAGAGATGGCA-GAATCCTTTCCA	0.8 kb
337	12276-L13219	Exon 26	4634-4635	TTCCGTCCTAAA-TGCTTGAGGACT	0.9 kb
166 #	12264-L13207	Exon 27	4730-4731	TGGGCATGTCCA-CCAAGTGAGGGA	2.1 kb
154 #	09934-L10393	Exon 28	4949-4950	TGGCCCAATGTT-CTGGAAGAAAGC	1.2 kb
425	12281-L13224	Exon 29	5141-5142	ATGCCCAATGTA-TCTAACGACCTC	2.7 kb
250 #	12271-L13214	Exon 30	5429-5430	ACCTGCAATGAA-TGCAAGCACCAT	0.6 kb
264	13429-L15124	Exon 31	5834-5835	CCGTTCTGCCTA-AACATCAAGCAG	
		stop codon	*7656-7658 (Exon 31)*		

Table 2b. *CREBBP*

Length (nt)	SALSA MLPA probe	CREBBP exon[a]	Ligation site NM_004380.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*798-800 (Exon 1)*		
346	09896-L10309	Exon 17	4123-4124	CCCAGAGTCATT-ACCTTTCCGGCA	
		stop codon	*8124-8126 (Exon 31)*		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

« 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.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Related SALSA MLPA probemixes

P313 CREBBP Contains probes for the *CREBBP* gene.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P333 EP300

- López M et al. (2016). First case report of inherited Rubinstein-Taybi syndrome associated with a novel EP300 variant. *BMC med genet*, 17(1), 97.
- López M et al. (2018). Rubinstein-Taybi 2 associated to novel *EP300* mutations: deepening the clinical and genetic spectrum. *BMC med genet*, 19(1), 36.
- Negri G et al (2016). From Whole Gene Deletion to Point Mutations of EP300-Positive Rubinstein–Taybi Patients: New Insights into the Mutational Spectrum and Peculiar Clinical Hallmarks. *Hum mutat*, 37(2), 175-183.
- Negri G et al. (2015). Clinical and molecular characterization of Rubinstein-Taybi syndrome patients carrying distinct novel mutations of the *EP300* gene. *Clin Genet.* 87:148-154.
- Tsai AC et al. (2011). Exon deletions of the *EP300* and *CREBBP* genes in two children with Rubinstein–Taybi syndrome detected by aCGH. *Eur J Hum Genet.* 19:43-9.
- Wincent J et al. (2016). *CREBBP* and *EP300* mutational spectrum and clinical presentations in a cohort of Swedish patients with Rubinstein–Taybi syndrome. *Mol Genet Genomic Med*, 4(1), 39-45.

P333 Product history	
Version	*Modification*
A4	One reference probe has been removed and two reference probes have been replaced.
A3	Three reference probes have been replaced and the length of two probes has been adjusted.
A2	One reference probe has been removed and QDX2 fragments have been added.
A1	First release.

Implemented changes in the product description

Version A4-02 – 29 November 2024 (02P)

- The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate.

Version A4-01 – 14 April 2020 (02P)

- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *EP300* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

More information: www.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mlpa.com (information & technical questions); order@mlpa.com (orders)
Phone	+31 888 657 200