Product Description SALSA® MLPA® Probemix P313-B3 CREBBP

To be used with the MLPA General Protocol.

Version B3. Compared to version B2 two reference probes have been replaced. For complete product history see page 7.

Catalogue numbers:
- **P313-025R:** SALSA MLPA Probemix P313 CREBBP, 25 reactions.
- **P313-050R:** SALSA MLPA Probemix P313 CREBBP, 50 reactions.
- **P313-100R:** SALSA MLPA Probemix P313 CREBBP, 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 P313 CREBBP is a **research use only (RUO)** assay for the detection of copy number variations in the *CREBBP* and *EP300* genes, which are associated with Rubinstein-Taybi syndrome (RSTS; OMIM 180849).

RSTS is a well-defined multiple congenital anomalies / intellectual disability syndrome characterised by postnatal growth deficiency, microcephaly, specific facial characteristics, broad thumbs and big toes, and intellectual disability. It occurs generally sporadic, and can be caused by a microdeletion of chromosome 16p13.3, or by a mutation in either CREB-binding protein (*CREBBP*) or the E1A-binding protein (*EP300*). Birth prevalence is 1 in 100.000–125.000.

Most individuals with RSTS have point mutations in the *CREBBP* or the *EP300* gene, most of which will not be detected by the MLPA technique. Partial or complete deletions and duplications of the *CREBBP* gene have also been described (Roelfsema et al. 2005). Please note that this P313 probemix is different from the probemix that was used by Roelfsema et al.

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 *CREBBP* and *EP300* exon numbering used in this P313-B3 CREBBP product description is the exon numbering from the RefSeq transcripts NM_004380.3 and NM_001429.4, which is identical to the LRG_1426 and LRG_1422 sequences, respectively. The exon numbering and NM_ sequence used have been retrieved on 11/2019. 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 P313-B3 CREBBP contains 46 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes 34 probes for the *CREBBP* gene, One probe for each exon and two probes for exons 1, 2, and 3, and three probes for the *EP300* gene, one probe for exon 1, 4, and 12. 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, 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 Rubinstein-Taybi 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 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 *CREBBP* and *EP300* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P313 CREBBP.
- 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.

***CREBBP* mutation database:** https://databases.lovd.nl/shared/genes/CREBBP. 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., a duplication of *CREBBP* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P313-B3 CREBBP

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*CREBBP*	*EP300*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 11230-L11913	8q21		
139	**CREBBP probe** 03088-L11381		**Exon 31**	
144	**CREBBP probe** 21107-L29531		**Exon 7**	
150	**CREBBP probe** 09898-L11194		**Exon 19**	
154	Reference probe 09431-L09680	11q13		
160	**CREBBP probe** 09903-L10316		**Exon 24**	
166	**CREBBP probe** 09890-L10303		**Exon 11**	
172 «	**CREBBP probe** 03087-L02487		**Exon 1**	
178	Reference probe 02865-L02617	17q11		
184	**CREBBP probe** 09909-L10322		**Exon 30**	
190	**CREBBP probe** 09891-L10304		**Exon 12**	
196	**CREBBP probe** 09902-L10315		**Exon 23**	
201 «	**EP300 probe** 09925-L11195			**Exon 1**
207	**CREBBP probe** 09882-L10295		**Exon 3**	
214	**CREBBP probe** 09894-L10307		**Exon 15**	
221	**CREBBP probe** 09904-L10317		**Exon 25**	
229	Reference probe 05508-L04931	10q11		
237	**CREBBP probe** 09895-L10308		**Exon 16**	
247	**CREBBP probe** 09887-L10300		**Exon 8**	
255	**CREBBP probe** 09899-L10312		**Exon 20**	
265	Reference probe 03270-L02707	3q29		
272	**CREBBP probe** 09897-L10310		**Exon 18**	
280	**CREBBP probe** 21108-L10313		**Exon 21**	
292	**CREBBP probe** 09883-L10296		**Exon 3**	
299	Reference probe 07127-L06736	2p22		
308	**CREBBP probe** 03086-L24217		**Exon 4**	
317	**CREBBP probe** 09906-L10319		**Exon 27**	
325	**CREBBP probe** 03085-L04948		**Exon 2**	
337	**CREBBP probe** 09885-L10298		**Exon 6**	
346	**CREBBP probe** 09896-L10309		**Exon 17**	
355	**EP300 probe** 09930-L10389			**Exon 12**
364	**CREBBP probe** 09884-L10297		**Exon 5**	
375	**CREBBP probe** 09889-L10302		**Exon 10**	
382	**CREBBP probe** 09892-L10305		**Exon 13**	
391	**CREBBP probe** 09905-L10318		**Exon 26**	
400	Reference probe 07991-L07772	7q21		
409	**CREBBP probe** 09893-L10306		**Exon 14**	
418	**CREBBP probe** 09907-L10320		**Exon 28**	
427 «	**CREBBP probe** 09880-L10293		**Exon 1**	
436	**CREBBP probe** 09901-L10314		**Exon 22**	
445	**EP300 probe** 09927-L10386			**Exon 4**
454	**CREBBP probe** 09881-L29528		**Exon 2**	
463	**CREBBP probe** 09908-L10321		**Exon 29**	
476	**CREBBP probe** 09888-L10301		**Exon 9**	
481 *	Reference probe 15490-L17330	18q12		
490 *	Reference probe 08614-L21726	12p12		

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

* New in version B3.
« 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. P313-B3 probes arranged according to chromosomal location

Table 2a. *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)*		
172 «	03087-L02487	Exon 1	798-799	AGCAGGTGAAAA-TGGCTGAGAACT	0.1 kb
427 «	09880-L10293	Exon 1	861-862	GCTCGCCCGGTT-TCTCGGCGAATG	29.1 kb
454	09881-L29528	Exon 2	1128-1129	CGAACAGTGCTA-ACATGGCCAGCC	0.2 kb
325	03085-L04948	Exon 2	1340-1341	AATGCTAACTTT-AACCAGACCCAC	39.8 kb
207	09882-L10295	Exon 3	1624-1625	CACAAGTCCATT-TGGACAGCCCTT	0.2 kb
292	09883-L10296	Exon 3	1767-1768	CCAACGTGCCAA-ATATGGTAAGTT	17.1 kb
308	03086-L24217	Exon 4	1854-1855	AAAAACGCAAAC-TGATACAGCAGC	1.5 kb
364	09884-L10297	Exon 5	2048-2049	CAAATCATCTCT-CATTGGAAGAAC	9.2 kb
337	09885-L10298	Exon 6	2171-2172	AACACAATTGGT-TCTGTTGGCACA	1.6 kb
144	21107-L29531	Exon 7	2404-2405	AGCAGGAGGAAT-AACAACAGATCA	0.5 kb
247	09887-L10300	Exon 8	2566-2567	CGGTGTAAGGAA-AGGCTGGCACGA	2.0 kb
476	09888-L10301	Exon 9	2698-2699	AGCCTATGCTAA-GAAAGTGGAAGG	0.6 kb
375	09889-L10302	Exon 10	2830-2831	TAAACAAGGCAT-CTTGGGGAACCA	0.5 kb
166	09890-L10303	Exon 11	2936-2937	CTGCCAGTGAAT-CGCATGCAAGTT	2.9 kb
190	09891-L10304	Exon 12	2967-2968	GGATGAATTCAT-TTAACCCCATGT	0.8 kb
382	09892-L10305	Exon 13	3122-3123	CCTCCGAACATG-ATGGGTGCACAC	3.1 kb
409	09893-L10306	Exon 14	3464-3465	ACTCAGCCATCA-ACTCCTGTGTCG	1.5 kb
214	09894-L10307	Exon 15	3787-3788	TGTGCTGGAAAT-GAAGACGGAGAC	1.4 kb
237	09895-L10308	Exon 16	3895-3896	TTCCCAAGTTAA-AGAAGAAACAGA	9.0 kb
346	09896-L10309	Exon 17	4123-4124	CCCAGAGTCATT-ACCTTTCCGGCA	1.0 kb
272	09897-L10310	Exon 18	4279-4280	CTGGCTCATGTT-CAACAATGCCTG	0.6 kb
150	09898-L11194	Exon 19	4487-4488	TACTACAGCTAT-CAGAATAGGTAA	5.5 kb
255	09899-L10312	Exon 20	4523-4524	AAGTGTTTCACA-GAGATCCAGGGC	2.2 kb
280	21108-L10313	Exon 21	10 nt after exon 21	CCGTAAGTATAT-AGCTATTTCTTT	4.3 kb
436	09901-L10314	Exon 22	4658-4659	AAGGAGTGTGGC-CGGAAGATGCAT	0.4 kb
196	09902-L10315	Exon 23	4741-4742	CTTGAAGAAAAC-TGGCAGACCTCG	4.5 kb
160	09903-L10316	Exon 24	4896-4897	CCAGCTCAGACA-AGACGGTGGAGG	0.7 kb
221	09904-L10317	Exon 25	4955-4956	GGGGAAATGTCT-GAATCTTTCCCA	1.1 kb
391	09905-L10318	Exon 26	5106-5107	ATCTGGATAGTA-TTCATTTCTTCC	1.9 kb
317 #	09906-L10319	Exon 27	5318-5319	AAAAAGATGCTG-GACAAGGCGTTT	0.6 kb
418	09907-L10320	Exon 28	5420-5421	CCCTATTTTGAA-GGTGATTTCTGG	4.3 kb
463	09908-L10321	Exon 29	5648-5649	TCCAATGACCTG-TCCCAGAAGCTG	0.4 kb
184	09909-L10322	Exon 30	5704-5705	CTTCGTGATCCA-CCTGCACGCTGG	3.8 kb
139	03088-L11381	Exon 31	8130-8131	GCTTGTAGCATT-GTGAGAGCATCA	
		stop codon	*8124-8126 (Exon 31)*		

Table 2b. *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	34.7 kb
445	09927-L10386	Exon 4	1541-1542	AAGAATGTCCTA-AACCACATGACA	20.2 kb
355	09930-L10389	Exon 12	2628-2629	ACCATGGACAGT-TGGCTCAACCTG	
		stop codon	*7656-7658 (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.

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.
« 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.

Related SALSA MLPA probemixes

P333 EP300 Contains probes for the *EP300* gene.

References

- Roelfsema JH et al. (2005) Genetic Heterogeneity in Rubinstein-Taybi Syndrome: Mutations in both CBP and EP300 genes cause disease. *Am J Hum Genet* 76:572-580.
- 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 P313 CREBBP

- Calì F et al. (2013). Multiplex ligation-dependent probe amplification detection of an unknown large deletion of the CREB-binding protein gene in a patient with Rubinstein-Taybi syndrome. *Genet Mol Res*, *12*(3), 2809-2815.
- Fadley MAM et al. (2012) Chromosomal 16p microdeletion in Rubinstein-Taybi syndrome detected by oligonucleotide-based array comparative genomic hybridization. *J of Med Case reports.* 6.1:1.
- Lee JS et al. (2015). Clinical and mutational spectrum in Korean patients with Rubinstein–Taybi syndrome: The spectrum of brain MRI abnormalities. *Brain Dev*, *37*(4), 402-408.
- 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.
- Pérez-Grijalba V et al. (2019). New insights into genetic variant spectrum and genotype–phenotype correlations of Rubinstein-Taybi syndrome in 39 CREBBP-positive patients. *Mol Genet Genom Med*.
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- Tsai ACH et al. (2011) Exon deletions of the EP300 and CREBBP genes in two children with Rubinstein–Taybi syndrome detected by aCGH. *Eur J of Hum Gen.* 19.1:43-49.
- Yu S et al. (2019). Clinical exome sequencing identifies novel CREBBP variants in 18 Chinese Rubinstein–Taybi Syndrome kids with high frequency of polydactyly. *Mol Genet Genom Med*, e1009.

P313 Product history	
Version	*Modification*
B3	Two reference probes have been replaced.
B2	One reference probe has been added and one replaced, in addition several lengths have been adjusted.
B1	Seven reference probes have been replaced, control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description

Version B3-02 — 29 November 2024 (02P)
- The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate.

Version B3-01 — 9 January 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 *CREBBP* and *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