Product Description SALSA® MLPA® probemix P340-B1 EHMT1

To be used with the MLPA General Protocol.

P340 Version B1. As compared to version A2, one *EHMT1* exon 26 and two reference probes have been removed, three reference probes have been replaced and several probe lengths have been adjusted. For complete product history see page 6.

Catalogue numbers:
- **P340-025R:** SALSA® MLPA® probemix P340 EHMT1, 25 reactions.
- **P340-050R:** SALSA® MLPA® probemix P340 EHMT1, 50 reactions.
- **P340-100R:** SALSA® MLPA® probemix P340 EHMT1, 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.

General Information: The SALSA MLPA Probemix P340 EHMT1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *EHMT1* gene. The *EHMT1* gene (27 exons) spans ~217 kb of genomic DNA and is located on chromosome 9q34.3, ~139 Mb from the p-telomere.

Kleefstra syndrome (KS) is characterized by developmental delay, intellectual disability, hypotonia and distinct facial features. Additional clinical features include congenital heart defects, cerebral abnormalities, urogenital defects and weight gain. The syndrome is caused by a microdeletion in chromosomal region 9q34.3 (in 85% of cases) or by a mutation in the *EHMT1* gene coding for euchromatin histone methyltransferase 1. The prenatal phenotype has not yet been characterized.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK47079/.

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/

Probemix content: The SALSA MLPA Probemix P340-B1 EHMT1 contains 42 MLPA probes with amplification products between 130 and 494 nt. This P340-B1 probemix contains one probe for each exon of the *EHMT1* gene, except for exon 1. Two probes are present for exons 2, 10 and 19. This probemix furthermore contains two probes for *ARRDC1-AS1* (previous name: *C9orf37*) which is located ~1 kb upstream of *EHMT1* exon 1, and one probe for the *CACNA1B* gene, which is located ~50 kb downstream of *EHMT1*. In addition, nine reference probes are included in this probemix, detecting nine different autosomal chromosomal locations. Complete probe sequences and 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)

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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 *EHMT1*. 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.

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 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 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 cause of genetic defects in the *EHMT1* gene are small (point) mutations, none of which will not be detected by using SALSA® MLPA® probemix P340 EHMT1.
- 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.

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 *EHMT1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA P340-B1 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*EHMT1*
64-105	Control fragments – see table in probemix content section for more information		
130 *	Reference probe 19551-L26105	2p13	
136 ¥	**EHMT1 probe** 21189-L16399		**Exon 2**
148	**EHMT1 probe** 14209-L15831		**Exon 16**
154 ¥	**EHMT1 probe** 20975-L15832		**Exon 7**
166	**EHMT1 probe** 14211-L15833		**Exon 18**
171 ¥	**EHMT1 probe** 20980-L29515		**Exon 23**
178 ¥ «	**EHMT1 probe** 20976-L29302		**Intron 1**
184 ¥ ¬ +	**CACNA1B probe** 21040-L29516		Downstream
196	**EHMT1 probe** 14215-L15837		**Exon 10**
202	**EHMT1 probe** 14216-L15838		**Exon 21**
208 ¥	**EHMT1 probe** 20977-L29517		**Exon 6**
214	Reference probe 08172-L08052	10p13	
221 ¥ ¬ «	**ARRDC1-AS1 probe** 20978-L29304		Upstream
226 ¥	**EHMT1 probe** 20979-L29200		**Exon 2**
232	**EHMT1 probe** 14220-L15842		**Exon 24**
240	Reference probe 06610-L10371	20q13	
247 ¥	**EHMT1 probe** 21002-L29902		**Exon 10**
256	**EHMT1 probe** 14223-L16398		**Exon 22**
266	**EHMT1 probe** 14225-L15847		**Exon 4**
274	Reference probe 10458-L11011	11q13	
280 ¥	**EHMT1 probe** 21190-L29300		**Exon 3**
292	**EHMT1 probe** 14227-L15849		**Exon 20**
299 ¥	**EHMT1 probe** 21003-L29306		**Exon 8**
311	**EHMT1 probe** 14229-L15851		**Exon 25**
319 *	Reference probe 11700-L12471	17q25	
328	**EHMT1 probe** 14230-L15852		**Exon 12**
337	Reference probe 10997-L11668	4q22	
346	**EHMT1 probe** 14231-L15853		**Exon 27**
355	**EHMT1 probe** 14722-L15843		**Exon 13**
364	Reference probe 11207-L11890	15q26	
373	**EHMT1 probe** 14232-L15854		**Exon 19**
382	**EHMT1 probe** 14233-L15855		**Exon 17**
391	**EHMT1 probe** 14234-L15856		**Exon 5**
401 ¥	Reference probe 07518-L29903	14q24	
409	**EHMT1 probe** 14723-L15857		**Exon 26**
427	**EHMT1 probe** 14236-L15858		**Exon 11**
436	**EHMT1 probe** 14237-L15859		**Exon 15**
445 ¬ «	**ARRDC1-AS1 probe** 14238-L15860		Upstream
463	**EHMT1 probe** 14239-L15861		**Exon 19**
472 ¥	**EHMT1 probe** 21188-L29305		**Exon 14**
481	**EHMT1 probe** 14241-L15863		**Exon 9**
494 *	Reference probe 17965-L29904	18q21	

* New in version B1 (from lot B1-0218 onwards).
¥ Changed in version B1 (from lot B1-0218 onwards). Small change in length, no change in sequence detected.
¬ Flanking probe. Included only to facilitate determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.
« 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 detects a second target site on 1q21.1. This second target site is present in the hg38 and hg19 genome builds but not in the hg18 build.

Note: The exon numbering used in this P340-B1 *EHMT1* product description is the exon numbering from the RefSeq transcript NM_024757.4, which is identical to the NG_011776.1. The exon numbering and NM

sequence used is from March 2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. P340 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*EHMT1* Exon	Ligation site NM_024757.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
221 ¥¬ «	20978-L29304	*ARRDC1-AS1* Exon 2	NR_122035.1 524-523 reverse	GAAACCTCAAAT-TCAGTCCATGCT	0.7 kb
445 ¬ «	14238-L15860	*ARRDC1-AS1* Exon 1	NR_122035.1 179-178 reverse	TCAATTTAGAAT-GAGACATGTGTT	1.0 kb
	EHMT1 Start Codon		*38-40 (exon 1)*		
178 ¥ «	20976-L29302	Intron 1	624 nt after exon 1	CGGACTCAAGTT-TATTTCCAAACC	91.3 kb
136 ¥	21189-L16399	Exon 2	15 nt before exon 2	CTGACGGCTGTT-GTTTCTCTCTAA	0.1 kb
226 ¥	20979-L29200	Exon 2	103-104	TGCTGTGTGAAA-ACCGAGCTGCTG	5.6 kb
280 ¥	21190-L29300	Exon 3	155-156	AAGGCTCAGCAG-AGAAACAGGCAG	11.7 kb
266	14225-L15847	Exon 4	704-705	AAGATCCCAGAG-AAGTTCGAGAAG	15.1 kb
391	14234-L15856	Exon 5	991-992	ATGTTTAAGAGC-ATAACTCATTCC	0.4 kb
208 ¥	20977-L29517	Exon 6	27 nt before exon 6	CTGATCCTGCCT-TGGGGTATACAC	8.5 kb
154 ¥	20975-L15832	Exon 7	Intron 6-1208	GTGGCTGATCAG-ATGGACGGGGAG	1.9 kb
299 ¥	21003-L29306	Exon 8	1306-1307	TCCAGCATTAAG-AAGAAATTTCTC	3.8 kb
481	14241-L15863	Exon 9	1491-1492	AGTTTCTCTGGA-CTCCCTGGATCT	4.7 kb
247 ¥	21002-L29902	Exon 10	1550-1551	GGTTGGCCAACG-GTCCAGATGTGC	0.1 kb
196	14215-L15837	Exon 10	1613-1614	GCCGGATGGAAA-CACCGAAGAGTC	12.4 kb
427	14236-L15858	Exon 11	1767-1768	GCTGTGTGAAGA-CCACCGGGGCCG	1.4 kb
328	14230-L15852	Exon 12	1856-1857	GTCAGCCCGAGA-GCAGCATCTCTC	1.4 kb
355	14722-L15843	Exon 13	2194-2195	GGGAAGGAAACC-TTGGAGAGCGCT	1.6 kb
472 ¥	21188-L29305	Exon 14	2255-2256	GCTTCCACCCAA-AGCAGCTGTACT	2.7 kb
436	14237-L15859	Exon 15	2338-2339	CCCAACTTCAAA-ATGGAGCACCAG	8.5 kb
148	14209-L15831	Exon 16	2435-2436	GCGCTAATATTG-ACACCTGCTCAG	8.0 kb
382	14233-L15855	Exon 17	2596-2597	GGCCACTACGAA-GTGGTCCAGTAC	2.0 kb
166	14211-L15833	Exon 18	2662-2663	GGAGGCTGGACA-CCCATGATCTGG	10.6 kb
373	14232-L15854	Exon 19	2794-2795	TCCGGCTGCGTG-GACATAGCCGAG	0.1 kb
463	14239-L15861	Exon 19	2854-2855	AACATCCACGGA-GACTCGCCACTG	1.5 kb
292	14227-L15849	Exon 20	2973-2974	CCTGCAGTGTGC-GAGCCTCAACTC	0.4 kb
202	14216-L15838	Exon 21	3197-3198	TGAACATCGACA-GAAATATCACTC	1.0 kb
256	14223-L16398	Exon 22	3249-3250	CTGCTCCTCCAG-CAACTGCATGTG	1.5 kb
171 ¥	20980-L29515	Exon 23	3313-3314	CGGCTCCTGCCA-GAGTTCAACATG	1.5 kb
232	14220-L15842	Exon 24	3415-3416	TTTTTCAGGGCA-AGGCTGCAGCTC	0.6 kb
311	14229-L15851	Exon 25	3524-3525	TGATTTCAGACT-CAGAAGCCGACG	16.3 kb
409	14723-L15857	Exon 26	3589-3590	GACGGGGAGGTT-TACTGCATCGAC	0.5 kb
346	14231-L15853	Exon 27	3795-3796	CAAAGGCAAGCT-CTTCAGCTGCCG	48.1 kb
	EHMT1 Stop Codon		*3932-3934 (exon 27)*		
184 ¥ ¬ +	21040-L29516	*CACNA1B* Exon 3		GTCGTCCTCACA-GGGTAGGCAAGC	

¥ Changed in version B1 (from lot B1-0218 onwards). Small change in length, no change in sequence detected.
¬ Flanking probe. Included only to facilitate determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.
« 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 detects a second target site on 1q21.1. This second target site is present in the hg38 and hg19 genome builds but not in the hg18 build.

Note: The exon numbering used in this P340-B1 EHMT1 product description is the exon numbering from the RefSeq transcript NM_024757.4, which is identical to the NG_011776.1. The exon numbering and NM

sequence used is from March 2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA® MLPA® probemixes

- P286 Human Telomere-11: Contains additional probes for the 9q subtelomeric region.

References

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

Selected publications using SALSA MLPA Probemix P340

- De Boer, A et al. (2018). EHMT1 mosaicism in apparently unaffected parents is associated with autism spectrum disorder and neurocognitive dysfunction. *Molecular Autism* 9:5. DOI 10.1186/s13229-018-0193-9.
- Nillesen, WM et al. (2011). Characterization of a novel transcript of the EHMT1 gene reveals important diagnostic implications for Kleefstra syndrome. *Human Mutation*. 32(7); 853-859.
- Kleefstra T et al. (2009). Further clinical and molecular delineation of the 9q subtelomeric deletion syndrome supports a major contribution of EHMT1 haploinsufficiency to the core phenotype. *Journal of Medical Genetics.* 2009 46(9):598-606.

P340 Product history	
Version	*Modification*
B1	One EHMT1 exon 26 and two reference probes have been removed, three reference probes have been replaced and several probe lengths have been adjusted.
A2	Control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description

Version B1-02 – 24 September 2021 (01P)
- A warning about a second target site was added to Table 1 and Table 2 for the flanking probe targeting *CACNA1B*.

Version B1-01 – 23 March 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).

Version 07 – 23 November 2015 (55)
- Product description adapted to a new lot (lot number added, new picture included).
- Exon numbering of the *EHMT1* gene has been changed according to NG_011776.1 on page 3 and 4.
- *C9orf37* has been renamed to *ARRDC1-AS1* according to HGNC (Hugo Gene Nomenclature Committee).

Version 06 (48)
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.

Version 05 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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