Product Description SALSA® MLPA® Probemix P336-B1 UBE3A

To be used with the MLPA General Protocol.

Version B1. For complete product history see page 8.

Catalogue numbers:
- **P336-025R:** SALSA MLPA Probemix P336 UBE3A, 25 reactions.
- **P336-050R:** SALSA MLPA Probemix P336 UBE3A, 50 reactions.
- **P336-100R:** SALSA MLPA Probemix P336 UBE3A, 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 P336 UBE3A is a **research use only (RUO)** assay for the detection of deletions or duplications in the *UBE3A* gene, which is associated with Angelman syndrome (AS).

AS is a maternally inherited neurodevelopmental disorder characterized by intellectual disability, ataxia, epilepsy, minimal speech, and characteristic behavioural features. Defects in the *UBE3A* gene on chromosome 15q11.2 is one of the causes of AS. The protein encoded by this gene is ubiquitin protein ligase E3A, a part of the ubiquitin protein degradation system. AS individuals fail to inherit a normal active maternal copy of *UBE3A*. *UBE3A* is subject to genomic imprinting, with predominant transcription of the maternal allele in brain.

Besides or along with mutations in the *UBE3A* gene, other known genetic causes of AS are maternal deletion of chromosome 15q11-q13, paternal chromosome 15 uniparental disomy, and 15q11 imprinting defects. Maternal duplications of 15q11-q13 lead to a distinct condition that often includes autism (Stewart et al. 2011, Hogart et al. 2009). An AS-like phenotype might be caused by aberrations in the *MTHFR* gene. Moreover, there has been evidence for linkage of 16p13 to autism (Liu et al. 2012, IMGSAC 2001).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1144/.

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 *UBE3A*, *MTHFR*, and *GABRB3* exon numbering used in this P336-B1 UBE3A product description is the exon numbering from the RefSeq transcripts NM_130838.1, NM_005957.4, and NM_021912.5 which are identical to the LRG_15, LRG_726, and NG_012836.1 sequences. The exon numberings and NM_ sequences used have been retrieved on 05/2020. 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 P336-B1 UBE3A contains 37 MLPA probes with amplification products between 129 and 454 nucleotides (nt). This includes 18 probes for the *UBE3A* gene, one probe for each exon, two probes for exons 5 and 10, and six probes upstream of the gene. Furthermore, this probemix also contains three probes for the *GABRB3* gene, targeting exons 2, 4, and 7, and four probes for the *MTHFR* gene, targeting exons 3, 5, 8, and 10. In addition, twelve 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 Angelman 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, a (small) part of the cause of genetic defects in the *UBE3A* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P336 UBE3A.
- 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.

***UBE3A* mutation database:** https://databases.lovd.nl/shared/genes/UBE3A. 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 *UBE3A* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P336-B1 UBE3A

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	1p36 MTHFR	15q11 UBE3A	15q12 GABRB3
64-105	Control fragments – see table in probemix content section for more information				
129	Reference probe 11622-L12379	10q25			
136 *	Reference probe 14277-L15947	11q14			
142	**UBE3A probe** 10883-L11553			**Exon 5**	
148	**MTHFR probe** 12083-L12971		**Exon 5**		
155 *	Reference probe 16253-L18545	19p13			
179	**UBE3A probe** 10882-L11552			**Exon 5**	
184	**GABRB3 probe** 10868-L11538				**Exon 4**
197	**UBE3A probe** 13727-L15208			**Upstream**	
203	**GABRB3 probe** 10873-L11543				**Exon 7**
208	Reference probe 09865-L08705	13q32			
215	**UBE3A probe** 10885-L11555			**Exon 7**	
220	**MTHFR probe** 12085-L14676		**Exon 10**		
226	Reference probe 13598-L15056	9q21			
232	**UBE3A probe** 13728-L15209			**Exon 3**	
244	**UBE3A probe** 10886-L14677			**Exon 8**	
250 ¥	**GABRB3 probe** 10866-L29479				**Exon 2**
258	**UBE3A probe** 01317-L14678			**Exon 9**	
265	**MTHFR probe** 12086-L13395		**Exon 3**		
283	**UBE3A probe** 10879-L11549			**Exon 1**	
292	**UBE3A probe** 13729-L15210			**Upstream**	
302 *	Reference probe 15881-L17974	2p16			
310	**UBE3A probe** 10884-L11554			**Exon 6**	
319	**MTHFR probe** 12087-L13396		**Exon 8**		
328	Reference probe 08543-L08544	3q24			
341	**UBE3A probe** 14083-L15682			**Exon 10**	
349	Reference probe 13442-L14897	17p13			
359 Ж	**UBE3A probe** 13731-SP0136-L15212			**Exon 4**	
365	**UBE3A probe** 13732-L16039			**Upstream**	
372	**UBE3A probe** 14012-L15214			**Exon 2**	
381 *	Reference probe 10693-L23477	6p12			
390	**UBE3A probe** 14490-L16067			**Upstream**	
400	Reference probe 07678-L06854	7p15			
409	**UBE3A probe** 13735-L15216			**Upstream**	
417 ¥	**UBE3A probe** 14489-L29480			**Upstream**	
427	Reference probe 08046-L07827	5p15			
436	**UBE3A probe** 14085-L15684			**Exon 10**	
454	Reference probe 08579-L08580	17q23			

* New in version B1.

¥ Changed in version B1. Minor alteration, no change in sequence detected.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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

Table 2. P336-B1 probes arranged according to chromosomal location

Table 2a. MTHFR

Length (nt)	SALSA MLPA probe	MTHFR exon[a]	Ligation site NM_005957.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*230-232 (Exon 2)*		
265	12086-L13395	Exon 3	15 nt before exon 3	CTCTCTTCAGAA-ACAAACCCCCTA	5.2 kb
148	12083-L12971	Exon 5	833-834	CGACATGGGCAT-CACTTGCCCCAT	1.8 kb
319	12087-L13396	Exon 8	1313-1314	CCTCTTCTACCT-GAAGAGCAAGTC	2.1 kb
220	12085-L14676	Exon 10	1634-1635	CTACTTAGAGTT-TTTCACTTCCCG	
		stop sodon	*2198-2200 (Exon 12)*		

Table 2b. GABRB3 and UBE3A

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		GABRB3	**NM_021912.5**		
		start codon	*65-67 (Exon 1)*		
250	10866-L29479	Exon 2	201-202	CGCCTAAGACCC-GACTTCGGGGGT	151.2 kb
184	10868-L11538	Exon 4	351-352	GGGATCCCTCTC-AACCTCACGCTT	53.7 kb
203	10873-L11543	Exon 7	40 nt before exon 7	TTAGTCTGCCAT-GTTGTTTCTCCA	1128.8 kb
		stop codon	*1484-1486 (Exon 9)*		
		UBE3A	**NM_130838.1**		
		start codon	*45-47 (Exon 1)*		
292	13729-L15210	Upstream	NM_000462.5; 132-133	GGCCTTTTCCCT-TCGCCAGGACCC	0.4 kb
417	14489-L29480	Upstream	NM_000462.5; 4 nt after exon 1	GACGACAGGTCA-GTGTTGCCGCGG	26.6 kb
197	13727-L15208	Upstream	NM_000462.5; 624-625	CAGAAGTTTGGC-GAAATATGGTAT	0.1 kb
409	13735-L15216	Upstream	NM_000462.5; 134 nt after exon 2	CTAACTACACTT-CCAAGACTGTAT	2.7 kb
365	13732-L16039	Upstream	NM_000462.5; 734-735	ATGTCACCGAAT-GGCCACAGCTTG	0.5 kb
390	14490-L16067	Upstream	NM_000462.5; 796-797	CAGGATGGAGAA-GCTGCACCAGTG	3.3 kb
283	10879-L11549	Exon 1	131 nt after exon 1	ATGGGAGATAGG-AACATACCTACT	29.8 kb
372	14012-L15214	Exon 2	252-253	AGAAAGGAGCAA-GCTCAGCTTACC	4.6 kb
232 #	13728-L15209	Exon 3	1157-1158	AATCACAATGAA-GAAGATGATGAA	10.6 kb
359 Ж	13731-SP0136-L15212	Exon 4	1664-1665; 1691-1692	GAAGGAGAACAA-27 nt spanning oligo-GAATTTTTTCAG	3.6 kb
179	10882-L11552	Exon 5	1807-1808	AACTGAGGGTCA-GTTTACTCTGAT	0.1 kb
142 #	10883-L11553	Exon 5	1896-1897	TCTACAGGAAGC-TAATGGGGAAAA	0.8 kb
310 #	10884-L11554	Exon 6	2019-2020	TCACTTTCCAGA-TATCACAGACAG	1.4 kb
215 #	10885-L11555	Exon 7	2196-2197	TTCATATGGTGA-CCAATGAATCTC	0.2 kb
244	10886-L14677	Exon 8	2336-2337	TCTGTTCTGATT-AGGTGAGGTACT	14.2 kb
258	01317-L14678	Exon 9	2368-2369	TCATTCATTTAC-AGATGAACAGAA	1.8 kb
341	14083-L15682	Exon 10	3348-3349	GTCTTGCAATGA-ACTGTTTCAGTA	0.4 kb
436	14085-L15684	Exon 10	3717-3718	TACTTAATCATA-CAGTAAGCTGAC	
		stop codon	*2601-2603 (Exon 10)*		

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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.

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.

Related SALSA MLPA probemixes

P339 SHANK3	Primary screening for Autism-spectrum disorder.
P343 Autism	Contains additional probes for the 15q13 region and the *UBE3A* gene.
P396 SHANK2	Primary screening for Autism-spectrum disorder.
ME028 PWS/AS	Primary screening for Prader-Willi and Angelman Syndrome (copy number & methylation).

References

- Hogart A et al. (2009). Chromosome 15q11–13 duplication syndrome brain reveals epigenetic alterations in gene expression not predicted from copy number. *J Med Genet*, 46(2), 86-93.
- International Molecular Genetic Study of Autism Consortium (IMGSAC). (2001). A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. *Am J Hum Genet*, 69(3), 570-581.
- Liu JY et al. (2012). Neuropathology of 16p13. 11 deletion in epilepsy. *PLoS One*, 7(4).
- 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.
- Stewart LR et al. (2011). High frequency of known copy number abnormalities and maternal duplication 15q11-q13 in patients with combined schizophrenia and epilepsy. *BMC med genet*, 12(1), 154.
- 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 P336 UBE3A

- Aguilera C et al. (2017). Novel intragenic deletions within the *UBE3A* gene in two unrelated patients with Angelman syndrome: case report and review of the literature. *BMC med genet*, 18(1), 137.
- Aguilera C et al. (2019). Identification of a de novo splicing variant in the Coffin–Siris gene, SMARCE 1, in a patient with Angelman-like syndrome. *Mol genet genom med*, 7(1), e00511.
- Beleza-Meireles A et al. (2011). Novel deletion encompassing exons 5-12 of the *UBE3A* gene in a girl with Angelman syndrome. *Eur J Med Genet.* 54(3):348-50.
- Beygo J et al. (2019). Update of the EMQN/ACGS best practice guidelines for molecular analysis of Prader-Willi and Angelman syndromes. *Eur J Hum Genet*, 27(9), 1326-1340.
- Cali F et al. (2010). Novel deletion of the E3A ubiquitin protein ligase gene detected by multiplex ligation-dependent probe amplification in a patient with Angelman syndrome. *Exp Mol Med.* 42(12):842-8.
- Calounova G et al. (2008). *Am J Med Genet A.* Molecular and clinical characterization of two patients with Prader-Willi syndrome and atypical deletions of proximal chromosome 15q. 146A(15):1955-62.

P336 Product history	
Version	*Modification*
B1	Four probes detecting 16p13 and the *MTHFR* mutation-specific probe have been removed, four reference probes have been replaced, and two probes have been adjusted in length.
A2	One reference probe has been removed and QDX2 fragments have been added.
A1	First release.

Implemented changes in the product description
Version B1-02 — 11 December 2024 (02P) - The term 'mental retardation' is considered outdated and was removed as synonym of 'intellectual disability'. *Version B1-01 — 29 May 2020 (02P)* - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *MTHFR* and *GABRB3* genes updated according to new version of

the NM_ reference sequences.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 07 – 08 November 2016 (55)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Ligation sites and exon numbers of the probes targeting the *UBE3A* and *GABRB3* genes have been updated according to NCBI NM_ reference sequences in Table 1 and 2.
- Changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various textual changes throughout the document.

Version 06 – 16 February 2016 (55)
- Correction on NM_000462.3 transcript variant number below Table 2b.
- Manufacturer's address adjusted.
- Updated link for "Database of Genomic Variants".

Version 05 (48)
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.

Version 04 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 03 (48)
- 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).
- Various minor textual and layout changes on page 1.

Version 02 (48)
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Tables have been numbered.
- Data analysis method has been modified.
- Exon numbering of the GABRB3 gene has been changed on page 3 and 4.
- Various minor textual changes on page 1.
- Remark on RefSeqGene standard and transcript variant added below Table 2.
- Ligation sites of the probes targeting the *GABRB3*, *AXIN1* and *UBE3A* genes updated according to new version of the NM_reference sequence.

Version 01 (44)
- Not applicable, new document.

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