

Instructions for Use

SALSA® MLPA® Probemix ME028 Prader-Willi/Angelman



See also the MS-MLPA General Protocol, the product descriptions of the SALSA® MLPA® Reagent Kit, SALSA® HhaI and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix ME028 Prader-Willi/Angelman product page on our website to find Certificates of Analysis and a list of related products.


Product Name	SALSA® MLPA® Probemix ME028 Prader-Willi/Angelman
Version	D1
Catalogue numbers	ME028-025R (25 reactions) ME028-050R (50 reactions) ME028-100R (100 reactions)
Basic UDI-DI	n.a.
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	Catalogue Numbers
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® HhaI	SMR50


Storage and Shelf Life

Recommended conditions		
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A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE  2797 COLOMBIA
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More Information:	
www.mrcholland.com	
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E-mail	info@mrcholland.com (information & technical questions); order@mrcholland.com (orders)
Phone	+31 888 657 200

Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to C1, two methylation-specific SNRPN probes were added in the D1 version. Additionally, one digestion control and one reference probe were replaced and two probes were adjusted in length.

1. Intended Purpose

The SALSA MLPA Probemix ME028 Prader-Willi/Angelman is an in vitro diagnostic (IVD)¹ or research use only (RUO) semi-quantitative manual assay² to be used with DNA isolated from human peripheral whole blood specimens. ME028 Prader-Willi/Angelman is intended for the detection of one or two copies of the 15q11-q13 region in combination with hypermethylation of the *SNRPN* and *MAGEL2* differentially methylated regions (DMRs) to confirm a clinical diagnosis and potential cause for Prader-Willi syndrome (PWS) or one or two copies of the 15q11-q13 region and hypomethylation of the DMRs to confirm a clinical diagnosis and potential cause for Angelman syndrome (AS). Additionally, the assay allows for the detection of duplications within the 15q11-q13 region to confirm a clinical diagnosis and potential cause for 15q11 duplication syndrome. In rare cases, this product can also be used for carrier testing of at-risk family members.

Methylation changes and copy number variations (CNVs) detected with ME028 Prader-Willi/Angelman should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. In the majority of patients, defects in the 15q11-q13 region are copy number changes, but point mutations can occur which will not be detected by MLPA. Point mutations in *UBE3A* can cause AS, it is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of the *UBE3A* gene.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

¹ Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

² To be used in combination with a SALSA MLPA Reagent Kit, Salsa Hhal and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, dissolved in 5 µl TE _{0.1} buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: <ul style="list-style-type: none"> • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	<ul style="list-style-type: none"> • Provided by user 	
Reference Samples (Required)	<ul style="list-style-type: none"> • Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and methylation status and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation and for baseline methylation determination. Derived from unrelated individuals from families without a history of Prader-Willi/Angelman/15q duplication syndrome. 	
No-DNA Control (Preferably)	<ul style="list-style-type: none"> • Provided by user • TE_{0.1} buffer instead of DNA • To check for DNA contamination 	
Positive Control Samples (Preferably)	<ul style="list-style-type: none"> • Provided by user, or 	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the [MS-MLPA General Protocol](#).

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	Function
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

[Coffalyser.Net](#) should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the [Coffalyser.Net Reference Manual](#) for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our [support portal](#).

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤ 0.10 .

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (15q11 region)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	2	Normal
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

The DNA sequences that are detected by the seven MAGEL2/SNRPN methylation-specific probes are imprinted and expected to be maternally methylated in healthy individuals; fully methylated in Prader-Willi patients and unmethylated in Angelman syndrome patients.

Expected Results of Methylation-Specific Probes

Methylation Ratio (MR)	Methylation Status
≥ 0.85	Fully methylated
≤ 0.05	Unmethylated
0.40 – 0.65	50% methylated / normal imprinted
All other values	Ambiguous

Possible Results of Digestion Control Probes

Methylation Ratio (MR)*	Digestion Status
≤ 0.05	Complete digestion
> 0.05	Incomplete digestion

* Signals ≤ 0.10 are displayed as intra ratio percentage by Coffalyser.Net. For more information see the [Coffalyser.Net Reference Manual](#).

Examples of MS-MLPA results obtained with this probemix and a detailed interpretation guide can be found on the ME028 product page on www.mrcholland.com.

6. Performance Characteristics

Study	Description
Expected values for copy numbers in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤ 0.10 , the ranges stated in the copy number table in the product description can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix ME028 Prader-Willi/Angelman in 46 samples from healthy individuals with normal copy number and 16 samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested with exception of the two 15q duplication syndrome samples. However, since only some probes fell below the cut-off FR in each samples, the correct genotype can be established.
Expected values for methylation in normal and affected populations	The cut-off values for ME028 Prader-Willi/Angelman were determined through a precision study using ME028-C1-1116 (see TD-VAL-08-P000 SALSA MS-MLPA and SALSA HhaI Verification and Validation Study Report). The cut-off values were validated using 46 samples from healthy individuals and seven positive samples. This resulted in 322 methylation ratios (MRs) obtained with the seven methylation-specific probes targeting the two imprinted regions (SNURF and MAGEL2 DMR) included in the probemix,

	establishing the range for methylation status in healthy individuals as presented in the product description.																												
Limit of detection	A study that evaluated the acceptable minimum and maximum amount of sample DNA revealed that the use of 50-250 ng of human DNA is the recommended input. The use of insufficient or too much sample DNA can affect performance																												
Interfering substances	<p>Impurities in the DNA sample can affect the MLPA reaction. To minimise this effect, see Sample quality section under Precautions and warnings of the MLPA General Protocol.</p> <p>SNPs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl, EDTA, Fe³⁺, heparin, hemoglobin and bilirubin) can affect the MS-MLPA reaction.</p> <p>A study using SALSA MLPA Probemix ME028 Prader-Willi/Angelman was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs/methylation status. For most probes, expected FRs/MRs (FRs/MRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.</p> <table><tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr><tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td><u>Copy number</u>: Expected FR for 477/504 measurements <u>Methylation</u>: Expected MR for 95/98 probes</td></tr><tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td><u>Copy number</u>: Expected FR for 495/504 measurements <u>Methylation</u>: Expected MR for 93/98 probes</td></tr><tr><td>Fe³⁺ (FeCl₃)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td><u>Copy number</u>: Expected FR for 534/540 measurements <u>Methylation</u>: Expected MR for 101/105 measurements</td></tr><tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td><u>Copy number</u>: Expected FR for 538/540 measurements <u>Methylation</u>: Expected MR for 102/105 measurements</td></tr><tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td><u>Copy number</u>: Expected FR for 540/540 measurements <u>Methylation</u>: Expected MR for 99/105 measurements</td></tr><tr><td>Bilirubin</td><td>Endogenous – blood sample</td><td>0.14 µg/µL</td><td><u>Copy number</u>: Expected FR for 538/540 measurements <u>Methylation</u>: Expected MR or 101/105 measurements</td></tr></table> <p>* Results are summarised for all probes across all five samples tested.</p> <p>EDTA, NaCl and FeCl₃ showed a bigger effect on copy number determination, as compared to heparin, hemoglobin and bilirubin. Regarding the methylation status, no interfering substance had a major effect on final ratios for the methylation-sensitive probes.</p> <p>To minimise variability across 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.</p>	Interferent	Source	Testing Concentration	Results*	EDTA	Exogenous – specimen collection tubes	1.5 mM	<u>Copy number</u> : Expected FR for 477/504 measurements <u>Methylation</u> : Expected MR for 95/98 probes	NaCl	Exogenous – DNA extraction	40 mM	<u>Copy number</u> : Expected FR for 495/504 measurements <u>Methylation</u> : Expected MR for 93/98 probes	Fe ³⁺ (FeCl ₃)	Exogenous – DNA extraction	1 µM	<u>Copy number</u> : Expected FR for 534/540 measurements <u>Methylation</u> : Expected MR for 101/105 measurements	Heparin	Exogenous – specimen collection tubes	0.02 U/mL	<u>Copy number</u> : Expected FR for 538/540 measurements <u>Methylation</u> : Expected MR for 102/105 measurements	Hemoglobin	Endogenous – blood sample	0.02 µg/µl	<u>Copy number</u> : Expected FR for 540/540 measurements <u>Methylation</u> : Expected MR for 99/105 measurements	Bilirubin	Endogenous – blood sample	0.14 µg/µL	<u>Copy number</u> : Expected FR for 538/540 measurements <u>Methylation</u> : Expected MR or 101/105 measurements
Interferent	Source	Testing Concentration	Results*																										
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Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.																												
Accuracy	Results of accuracy are derived from trueness and precision studies. Trueness: previously genotyped samples were tested and found to have the expected results. Precision: results are not affected by operator, day, or laboratory site																												
Clinical validity*	<p><u>Prader-Willi syndrome</u>: 70-75% of cases are from paternal deletion of 15q11-q13, 25-30% maternal uniparental disomy (mUPD), 1% imprinting defect, and rare occurrences of a balanced translocation with breakpoint in the <i>SNURF-SNRPN</i> locus and deletion of <i>SNORD116</i> (Clinical utility gene card for Prader-Willi). Small deletions of <i>PWRN2</i> can also lead to PWS but cannot be detected by ME028 Prader-Willi/Angelman. As these deletions are rare (<1%) and ME028 Prader-Willi/Angelman detects all other molecular causes of PWS, the diagnostic sensitivity for PWS is >99%.</p> <p><u>Angelman syndrome</u>: 75% of cases are from a maternal deletion of 15q11-q13, 1-2% paternal uniparental disomy (pUPD), 3% imprinting defect, 5-10% variants in the <i>UBE3A</i> gene, and 10-15% unknown (Clinical utility gene card for Angelman). ME028 Prader-Willi/Angelman can detect deletions, UPD and imprinting defects. Point mutations in <i>UBE3A</i> cannot be detected and it is recommended to use ME028 Prader-Willi/Angelman in combination with sequence analysis for the <i>UBE3A</i> gene. Therefore, the diagnostic sensitivity for AS is ~80%.</p>																												

	<p><u>15q duplication syndrome</u>: Only maternally derived gains of the 15q11.2-q13.1 chromosome are causing 15q duplication syndrome (GeneReviews). Since ME028 Prader-Willi/Angelman can detect gains of 15q11 as well as determine the parent of origin via the methylation status, it can detect 100% of 15q duplication syndrome cases.</p> <p>*(Based on a 2000-2024 literature review)</p>
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Summary of Safety and Performance (SSP)

The SSP is available in the European database on medical devices (Eudamed), <https://ec.europa.eu/tools/eudamed>, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Hhal site	% methylated in normal blood-derived DNA; imprinted allele	Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
-		15q11.2	NIPA1	Exon 3	214.0 kb	154	02018-L00865	‡
-		15q11.2	TUBGCP5	Exon 8	964.5 kb	436	20702-L29063	‡
-		15q11.2	MKRN3	Exon 1	77.5 kb	172	20688-L28510	
-		15q11.2	MAGEL2	Exon 1	4.1 kb	418	11155-L29062	
+	50%	15q11.2	MAGEL2:TSS-DMR	-	38.1 kb	232	20701-L28529	
-		15q11.2	NDN	Exon 1	1.1 Mb	427	04026-L29645	
-		15q11.2	SNRPN	Upstream (Exon u1B)	6.1 kb	288	15261-L16736	
-		15q11.2	SNRPN	Upstream (Exon u1B)	64.9 kb	239	20692-L15415	
-		15q11.2	SNRPN	Upstream (Intron u2)	12.3 kb	278	12179-L13382	
-		15q11.2	SNRPN	Upstream (Intron u2)	13.4 kb	270	12182-L28519	
-		15q11.2	SNRPN	Upstream (Exon u5)	0.6 kb	256	20694-L28518	
-		15q11.2	SNRPN	Upstream (Exon u5)	33.8 kb	391	12477-L13519	
+	50%	15q11.2	SNRPN SNURF:TSS-DMR	-	0.1 kb	250	11181-L13997	
+	50%	15q11.2	SNRPN SNURF:TSS-DMR	-	0.3 kb	178	04106-L13905	
+	50%	15q11.2	SNRPN SNURF:TSS-DMR	-	0.3 kb	190	04104-L04294	
+	50%	15q11.2	SNRPN SNURF:TSS-DMR	-	0.5 kb	142	20687-L31784	¥ Ð
+	50%	15q11.2	SNRPN SNURF:TSS-DMR	-	0.7 kb	333	22586-L31906	*
+	50%	15q11.2	SNRPN SNURF:TSS-DMR	-	11.3 kb	443	22587-L31786	*
-		15q11.2	SNRPN	Exon 3	8.3 kb	294	01318-L13088	
-		15q11.2	SNRPN	Exon 6 (7)	75.7 kb	409	11177-L28521	
-		15q11.2	SNRPN SNORD116-1	-	24.4 kb	214	12719-L28514	
-		15q11.2	SNRPN SNORD116-11	-	15.9 kb	472	12721-L13796	
-		15q11.2	SNRPN SNORD116-23	-	247.8 kb	326	20697-L28525	
-		15q11.2	UBE3A	Exon 12 (10)	20.3 kb	355	02034-L12925	
-		15q11.2	UBE3A	Exon 7 (5)	11.1 kb	301	12082-L28520	
-		15q11.2	UBE3A	Exon 6 (4)	4.2 kb	160	04620-L00863	
-		15q11.2	UBE3A	Exon 5 (3)	29.8 kb	195	20689-L28513	
-		15q11.2	UBE3A	Exon 4 (2)	33.5 kb	373	10878-L11548	
+	0%	15q11.2	UBE3A	Exon 1	252.8 kb	184	19804-L28512	
-		15q12	ATP10A	Exon 15	171.9 kb	364	20695-L28523	
-		15q12	ATP10A	Upstream (Exon 1)	684.3 kb	226	20691-L28515	Ø
-		15q12	GABRB3	Exon 9	19.6 kb	220	01315-L00868	
-		15q12	GABRB3	Exon 7	1.4 Mb	382	10874-L11544	ω
-		15q13.1	OCA2	Exon 23	187.1 kb	137	20700-L28528	
-		15q13.1	OCA2	Exon 3	1.2 Mb	317	20698-L28526	
-		15q13.1	APBA2			202	01314-L00867	→ ω
+	0%	2q	Digestion Control			342	20703-L31907	¥ π
+	0%	8p	Digestion Control			461	22386-L09311	* π
-		1q	Reference			400	13588-L24039	
-		4q	Reference			310	14480-L16200	
-		5p	Reference			244	08051-L07832	
-		5q	Reference			129	18709-L26847	
-		6q	Reference			347	18474-L29176	
-		8p	Reference			481	19033-L24843	
-		10p	Reference			452	19636-L26295	
-		10q	Reference			264	07630-L17091	*
-		11q	Reference			166	08020-L07801	
-		12q	Reference			208	07404-L07051	
-		17q	Reference			148	08372-L08226	

Nomenclature according to Beygo et al. (2019) EMQN/ACGS best practice guidelines and Monk et al. (2018) Recommendations for a nomenclature system for reporting methylation aberrations in imprinted domains.

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

The exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of one probe with a target at the edge of or slightly outside the coding region, is altered. The exon numbering from the previous version of this product description is disclosed between brackets.

Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe changes

- * New probes.
- ¥ Probes changed in this product version. Minor alteration, no change in sequence detected.

Probe warnings

- Ø This probe targets a sequence outside of the known coding region. Copy number alterations of only this probe are of unknown clinical significance.
- ⊖ Be cautious when interpreting results if the probe signal of these two probes in the digested reaction is more than 15% lower than expected. The lower signal in digested reactions of these two probes indicates the use of an excess of HhaI enzyme or the use of an enzyme preparation that is unsuitable for MS-MLPA such as HhaI enzymes that are resistant to heat inactivation.
- π Digestion control: warns for insufficient digestion. Upon digestion, these probes should not give a signal. The MR of these probes should be checked to ensure digestion was complete.
- ⊕ This probe is sensitive to overdigestion, which can occur for example in case of lower ligation-digestion temperature (<48°C).
- This probe is outside the common PWS/AS region. The size of the region showing an aberrant copy number will differ between different PWS/AS patients.
- ‡ The chromosomal order of these probes differs between the hg18 and hg38 genome builds. We used the order based on hg38, as referenced by Jian et al. (2008).

Problemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product:** none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Sequence changes within a HhaI site can interfere with HhaI digestion and may result in a false positive MR. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.

4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. Results of methylation-specific probes tested on chorionic villi samples (CVS) might not reflect the actual epigenetic constitution of the fetus because the locus of interest might not have reached its final imprinting status in CVS. Therefore, only copy numbers can be determined when MS-MLPA is used on DNA extracted from CVS. DNA extracted from prenatal samples is **for research use only**.
6. The six methylation-specific probes targeting the *SNRPN* gene are located very close to each other. It is expected that all six probes provide similar results. We recommend using the median methylation status of these *SNRPN* probes to determine the methylation status of the *SNRPN* locus and to disregard aberrant methylation detected by a single *SNRPN* methylation-specific probe.
7. For 15q11 duplication syndrome patients, a duplication in copy number should first be verified before methylation status is assessed. The methylation status in 15q11 duplication patients will not follow the cut-off range for MR ratios. If a duplication is maternally inherited the ratios of imprinted methylation probes are expected to be ~0.7, and if the duplication is paternally inherited the ratios of imprinted methylation probes are expected to be ~0.3.

Technique-specific precautions

See the [MS-MLPA General Protocol](#).

8. Limitations

Problemix-specific limitations

1. No discrimination between UPD and imprinting defects can be made with this problemix. For this, it is necessary to perform microsatellite analysis in the patient and parents.
2. A methylation-specific probe targets a single specific HhaI site in a CpG island; if methylation is absent for a particular CpG-site, this does not necessarily mean that the whole CpG island is unmethylated.

Technique-specific limitations

See the [MS-MLPA General Protocol](#).

9. References Cited in this IFU

1. Beygo J et al. (2019). Update of the EMQN/ACGS best practice guidelines for molecular analysis of Prader-Willi and Angelman syndromes. *European journal of human genetics* : EJHG.
2. Monk D et al. (2018). Recommendations for a nomenclature system for reporting methylation aberrations in imprinted domains. *Epigenetics*. 13:117-21.
3. Jiang Y et al. (2008). Genomic analysis of the chromosome 15q11-q13 Prader-Willi syndrome region and characterization of transcripts for GOLGA8E and WHCD1L1 from the proximal breakpoint region. *BMC Genomics*. 9:50.

Implemented changes in the product description*Version D1-04 – 27 March 2025 (03S)*

- The product description has been updated to a new template.
- Intended purpose updated to specify that assay is manual and prenatal samples removed.
- Exon numbering of UBE3A probes (with exception of 19804-L28512) and SNRPN probes 15261-L16736, 20692-L15415, 12179-L13382, 12182-L28519, 20694-L28518, 12477-L13519 and 11177-L28521 updated according to MANE.
- SNVs rs541877352 and rs189040948 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Salt warnings have been removed from NDN probe 04026-L29645, MAGEL2 probes 11155-L29062 and 20701-L28529, ATP10A probe 20691-L28515, and UBE3A probe 19804-L28512.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Product is now IVDR certified.

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