Product Description
SALSA® MS-MLPA® Probemix ME033-A1 TNDM

To be used with the MS-MLPA General Protocol.

Version A1
For complete product history see page 9.

Catalogue numbers:
- **ME033-025R:** SALSA MS-MLPA Probemix ME033 TNDM, 25 reactions.
- **ME033-050R:** SALSA MS-MLPA Probemix ME033 TNDM, 50 reactions.
- **ME033-100R:** SALSA MS-MLPA Probemix ME033 TNDM, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, SALSA HhaI (SMR50) 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.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MS-MLPA General Protocol before use: www.mrcholland.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.

In several No DNA reactions performed on this probemix, MRC Holland has observed a series of non-specific peaks. These peaks appeared very sporadically and we have not yet been able to establish the cause. However, we found that the amount and height of these peaks is greatly reduced by not spinning down your MLPA reactions in between the ligation and PCR reaction. The non-specific peaks are not expected to influence results. Please notify us if you still regularly observe these peaks: info@mrcholland.com.

General information
The SALSA MS-MLPA Probemix ME033 TNDM is a **research use only (RUO)** assay for the detection of aberrant methylation of one or more sequences of the *PLAGL1* gene on chromosomal region 6q24. This probemix can also be used to detect deletions/duplications in the *PLAGL1* gene; the chromosomal regions 6p22 and 6q24, as well as the chromosomal region 11p15.

Genomic imprinting is the monoallelic expression of genes, dependent on the parental origin of the chromosome. It plays a role in growth and development. Imprinting disorders like Transient Neonatal Diabetes Mellitus (TNDM) originate from a disturbance in this monoallelic expression by disruption or epimutation of imprinted genes (Ishida et al. 2013).

TNDM is a form of diabetes that occurs in infants and is characterised by severe intra-uterine growth retardation, hyperglycemia, dehydration and absence of ketoacidosis.

Three different genetic mechanisms have been described as major causes of TNDM (Mitchell et al. 2010):

1. Paternal uniparental disomy of chromosome 6. This accounts for approximately ~40% of the 6q24-related TNDM cases and can be detected by an absence of methylation of the three *PLAGL1* promoter region probes that have an HhaI site. These three probes target a maternally imprinted genomic area: the maternal allele is methylated, while the paternal allele is unmethylated in normal control samples. As compared to reference probes that do not contain an HhaI site, the signal of the MS-MLPA probes in imprinted regions is reduced by 50% upon HhaI digestion in DNA samples from normal individuals.

While it is reduced in TNDM cases, due to the inheritance of two unmethylated paternal alleles and no imprinted maternal allele.

2. Duplication of 6q24 paternal allele. This accounts for ~30% of the 6q24-related TNDM cases and can be detected by a copy number change of the *PLAGL1* specific probes and other 6q24 probes.
3. Hypomethylation of the maternal *PLAGL1* differentially methylated region. This accounts for approximately ~30% of the 6q24-related TNDM cases and can be detected by a methylation change of the three *PLAGL1* promoter region probes that have an HhaI site. Approximately half of the hypomethylation cases are due to a defect *ZFP57* gene. The four *ZFP57*-specific MLPA probes in ME033 detect copy number changes of *ZFP57*. Recessive mutations of *ZFP57* have been identified in ~10% of all TNDM patients.

Copy number probes for two other genes are included in this probemix because of their involvement in TNMD: *INS* and *KCNJ11* (11p15). Recessive loss of function mutations in the *INS* gene have been reported in several patients with TNDM (Støy et al. 2021), whereas activating mutations in *KCNJ11* have been reported as a possible cause of TNDM (Gloyn et al. 2006). Additionally probes for *ZC2HC1B* (6q24, downstream of *PLAGL1*) and several other genes are included to determine copy numbers of the 6q24 region.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1534/.

This SALSA MS-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
Matched Annotation from NCBI and EMBL-EBI (MANE): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *HIVEP2*, *INS*, *KCNJ11*, *NMBR*, *PEX3*, *PLAGL1*, *SASH1*, *ZC2HC1B* and *ZFP57* exon numbering used in this ME033-A1 TNDM product description is the exon numbering from the MANE project (release version 1.0) based on the MANE Select transcripts NM_006734.4, NM_000207.3, NM_000525.4, NM_002511.4, NM_003630.3, NM_001317162.2, NM_015278.5, NM_001013623.3 and NM_001109809.5, respectively, which can be found in Tables 1 and 2. The *NMBR* and *PLAGL1* exon numbering have changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version A1-04 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select transcripts for these genes. The *LINC01625*, *HYMAI and STXBP5-AS1* exon numbering is derived from the NR_033919.1, NR_002768.3 and NR_034115.1 sequences, respectively.

As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date. Please note that exon numbering for the same gene might be different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
The SALSA MS-MLPA Probemix ME033-A1 TNDM contains 39 (MS-)MLPA probes with amplification products between 130 and 436 nucleotides (nt). Three MS-MLPA probes contain an HhaI recognition site and provide information on the methylation status of the PLAGL1_TSS_alt-DMR. All probes present will also give information on copy number changes in the analysed sample. In addition, ten reference probes are included that are not affected by HhaI digestion and detect genes located outside the *PLAGL1* gene, 6p22, 6q24 and 11p15 regions. Also, two digestion control probes are included in this probemix indicating whether or not restriction endonuclease digestion in the MS-MLPA reaction was complete. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.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 MS-MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MS-MLPA technique
The principles of the MS-MLPA technique (Nygren et al. 2005, Schouten et al. 2002) are described in the MS-MLPA General Protocol (www.mrcholland.com).

MS-MLPA technique validation
Internal validation of the MS-MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MS-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.

Results of MS-MLPA are highly dependent on the HhaI enzyme used. HhaI enzymes that are resistant to heat inactivation are NOT compatible with the MS-MLPA technique and will give aberrant results. These include, but may not be limited to, Thermo Fisher Scientific enzymes HhaI, ANZA 59 HhaI, and FastDigest HhaI. We recommend using SALSA HhaI enzyme (SMR50) as this restriction enzyme has been validated for use with MS-MLPA by MRC Holland.

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 MS-MLPA General Protocol.

The results of methylation-specific MLPA probes tested on chorionic villi samples (CVS) might not reflect the actual epigenetic constitution of the foetus. This is because the locus of interest might not have reached its final imprinting status in CVS (Paganini et al. 2015). Furthermore, Paganini et al. demonstrated that the rate at which the imprinting is set may differ between, and even within, loci. Consequently, the use of this product on CVS samples should involve examining and validating the methylation status of each individual MS-MLPA probe.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MS-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. When selecting reference samples, please note that methylation patterns may vary between tissue types and even age groups! Reference samples should be derived from different unrelated individuals who are from families without a history of Transient Neonatal Diabetes Mellitus. More information regarding the selection and use of reference samples can be found in the MS-MLPA General Protocol (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive

control DNA samples 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.mrcholland.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 copy number results

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

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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 MS-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.
- Digestion Control Probes. The target sequences of the digestion control probes are unmethylated in most blood-derived DNA samples. The signals of the digestion control probes should be gone upon complete digestion by HhaI.
- mRNA levels. We have no data showing that methylation detected by a particular probe indeed influences the corresponding mRNA levels.

- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- 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: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

NOTE: In case digestion control probes are not fully digested (>0.05), please contact info@mrcholland.com for more information.

ME033 specific note:
- Pay extra attention to the results of the digested reactions in case the undigested ratios of the probes at 142 nt (*PLAGL1*; 18458-L25628) and 227 nt (*PLAGL1*; 15755-L25779) are different from the probe at 157 nt (*PLAGL1*; 18460-L26610).

Limitations of the procedure
- MS-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 MS-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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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.
- An MS-MLPA 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!
- Rare cases are known in which apparent methylation as detected by an MS-MLPA probe proved to be due to a sequence change in or very near the HhaI site.
- No discrimination between uniparental disomy and imprinting defects can be made with this probemix. For this, it is necessary to perform microsatellite analysis in the patient and parents.

Confirmation of results
Confirmation of methylation status can be performed with another technique, such as MSP (methylation-specific PCR), pyrosequencing, digestion-based PCR assays, etc. 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 MS-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.

LOVD mutation database
https://databases.lovd.nl/shared/genes. 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 SNVs and unusual results (e.g., a duplication of *ZFP57* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MS-MLPA Probemix ME033-A1 TNDM

Length (nt)	SALSA MLPA probe	HhaI site	% methylated in normal blood-derived DNA	% expected signal reduction	Chromosomal position (hg18)		
					Reference	*PLAGL1*	Other region
64-105	Control fragments – see table in probemix content section for more information						
130	Reference probe 03896-L00020	-			11q		
136	**ZC2HC1B probe** 18457-L23634	-					6q24
142 Δ «	**PLAGL1 probe** 18458-L25628	+	50%	50%		PLAGL1_TSS_alt-DMR (Exon 1)	
148	**ZC2HC1B probe** 18459-L23636	-					6q24
157 «	**PLAGL1 probe** 18460-L26610	+	50%	50%		PLAGL1_TSS_alt-DMR (Exon 1)	
166	Reference probe 14281-L15951	-			15q		
174 π	**Digestion control probe** 18383-L24737	+	0%	100%	13q		
178	**STXBP5-AS1 probe** 18462-L23639	-					6q24
190	**ZC2HC1B probe** 18464-L23641	-					6q24
196	Reference probe 10975-L11646	-			14q		
202	**ZC2HC1B probe** 18465-L23642	-					6q24
208	**INS probe** 19503-L17352	-					11p15
215	**PLAGL1 probe** 18466-L23643	-				Exon 4	
220	Reference probe 06863-L06457	-			9p		
227 Δ «	**PLAGL1 probe** 15755-L25779	+	50%	50%		PLAGL1_TSS_alt-DMR (Exon 1)	
232	**INS probe** 15500-L25657	-					11p15
240	**PLAGL1 probe** 18335-L25631	-				Exon 8	
247	**LINC01625 probe** 19506-L23654	-					6q24
253	**SASH1 probe** 19507-L23651	-					6q24
265	**HIVEP2 probe** 18468-L23645	-					6q24
274	Reference probe 06439-L05965	-			3p		
283	Reference probe 04881-L04265	-			1p		
292	**ZFP57 probe** 18469-L23646	-					6p22
301 «	**KCNJ11 probe** 18470-L23647	-					11p15
310	**PLAGL1 probe** 18337-L23250	-				Exon 7	
319	**ZFP57 probe** 18471-L25633	-					6p22
328	**PLAGL1 probe** 18472-L25634	-				Exon 6	
337	**ZFP57 probe** 18473-L23650	-					6p22
346 «	**KCNJ11 probe** 19505-L23644	-					11p15
357 « π	**Digestion control probe** 02801-L25300	+	0%	100%	6q		
364	Reference probe 06969-L25635	-			17p		
373	**PLAGL1 probe** 18340-L23253	-				Exon 3	
382	**NMBR probe** 18476-L23653	-					6q24
391	**INS probe** 19504-L25656	-					11p15
400	Reference probe 17960-L22873	-			18q		
409	**ZFP57 probe** 18478-L23655	-					6p22
418	**PEX3 probe** 18479-L23656	-					6q24
427	Reference probe 18381-L23252	-			7p		
436	Reference probe 10093-L10517	-			8q		

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

« 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.

π Digestion control: warns for insufficient digestion. Upon digestion, this probe should not give a signal.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. ME033-A1 target probes arranged according to chromosomal location

Table 2a. Chromosome 6

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	HhaI site	Ligation site	Distance to next probe
		ZFP57	-	**NM_001109809.5**	
319	18471-L25633	Exon 5	-	1737-1736, reverse	2.6 kb
292	18469-L23646	Exon 4	-	733-732, reverse	0.4 kb
337	18473-L23650	Exon 3	-	67 nt after exon 3, reverse	1.1 kb
409	18478-L23655	Exon 2	-	492-493	110.1 **M**b
247	19506-L23654	*LINC01625* - Exon 4	-	NR_033919.1; 403-402, reverse	2.6 **M**b
382	18476-L23653	*NMBR* - Exon 4 (3)	-	NM_002511.4; 1549-1548, reverse	698.8 kb
265	18468-L23645	*HIVEP2* - Exon 5	-	NM_006734.4; 604-605	710.3 kb
418	18479-L23656	*PEX3* - Exon 11	-	NM_003630.3; 1203-1204	379.3 kb
		ZC2HC1B		**NM_001013623.3**	
148	18459-L23636	Exon 1	-	39-40	22.2 kb
190	18464-L23641	Exon 4	-	354-355	16.4 kb
136	18457-L23634	Exon 6	-	632-633	35.1 kb
202	18465-L23642	Exon 8	-	805-806	3.0 kb
		PLAGL1		**NM_001317162.2**	
240	18335-L25631	Exon 8 (9)	-	2382-2381, reverse	6.9 kb
310	18337-L23250	Exon 7 (8)	-	846-845, reverse	12.5 kb
328	18472-L25634	Exon 6 (7b)	-	366-365, reverse	5.7 kb
215	18466-L23643	Exon 4 (5)	-	24 nt before exon 4 (5), reverse	2.7 kb
373	18340-L23253	Exon 3 (4)	-	241-240, reverse	38.9 kb
157 «	18460-L26610	*PLAGL1* - Exon 1 (2); *HYMAI* – Exon 1	+	NM_001317162.2; 286 nt after exon 1 (2); NR_002768.3; 456-457	0.2 kb
227 Δ «	15755-L25779	*PLAGL1* - Exon 1 (2); *HYMAI* – Exon 1	+	NM_001317162.2; 126 nt after exon 1 (2); NR_002768.3; 296-297	0.2 kb
142 Δ «	18458-L25628	*PLAGL1* - Exon 1 (2); *HYMAI* – Exon 1	+	NM_001317162.2; 111-110, reverse; NR_002768.3; 111-110, reverse	3.2 **M**b
178	18462-L23639	*STXBP5-AS1* - Exon 2	-	NR_034115.1; 297-296, reverse	1.4 **M**b
253	19507-L23651	*SASH1* - Exon 20	-	NM_015278.5; 4119-4120	

Table 2b. Chromosome 11

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	HhaI site	Ligation site	Distance to next probe
		INS		**NM_000207.3**	
232	15500-L25657	Exon 3	-	10 nt after exon 3	1.0 kb
208	19503-L17352	Exon 2	-	233-232, reverse	0.4 kb
391	19504-L25656	Exon 1	-	37-38	15.2 **M**b

		KCNJ11		**NM_000525.4**	
346 «	19505-L23644	Exon 1	-	1623-1622, reverse	1.1 kb
301 «	18470-L23647	Exon 1	-	509-508, reverse	

[a] See section Exon numbering on page 2 for more information.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

« 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 3. Sequences detected by the ME033-A1 probes

Length (nt)	SALSA MS-MLPA probe	Partial sequence with HhaI site
142	**PLAGL1 probe** 18458-L25628	GTGCCACCTCCGCGG-CCATGACGGCGACCCGGGGAAGCGC
157	**PLAGL1 probe** 18460-L26610	ACCCGATTCTTACAA-CCTGGCGCTCTAACC
227	**PLAGL1 probe** 15755-L25779	TTTGCCGCGCCGCCT-ACGTGCGGGTCCGGG

The HhaI sites are marked with grey. Ligation sites are marked with –. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

ME032 UPD7-UPD14	Contains several probes for imprinted regions on chromosomes 7 and 14.
ME034 Multi-locus Imprinting	Contains probes for regions implicated in multi-locus imprinting disturbances on chromosomes 6, 7, 11, 14, 15, 19 and 20.
P117 ABCC8	Contains probes for the *ABCC8* gene, associated with familial hyperinsulinemic hypoglycemia 1 (HHF1).
P241 MODY Mix 1	Contains probes for the *HNF1A, HNF1B, HNF4A* and *GCK* genes, associated with Maturity-Onset Diabetes of the Young (MODY).
P357 MODY Mix 2	Contains probes for the *KLF11, NEUROD1, PAX4, CEL, INS, PDX1* and *HNF1B* genes, associated with Maturity-Onset Diabetes of the Young (MODY).

References

- Gloyn AL et al. (2006). KCNJ11 activating mutations are associated with development delay, epilepsy and neonatal diabetes syndrome and other neurological features. *Eur J Hum Genet*. 14:824-830.
- Ishida M et al. (2013). The role of imprinted genes in humans. *Mol Aspects Med*. 34:826-840.
- Mitchell BD et al. (2010). Genomic imprinting in diabetes. *Genome Med*. 2:55.
- Nygren AO et al. (2005). Methylation-specific MLPA (MS-MLPA): simultaneous detection of CpG methylation and copy number changes of up to 40 sequences. *Nucleic Acids Res*. 33:e128.
- Paganini L et al. (2015). Beckwith-Wiedemann syndrome prenatal diagnosis by methylation analysis in chorionic villi. *Epigenetics*. 10:643-649.
- 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.

- Støy J et al. (2021). In celebration of a century within insulin – Update of insulin gene mutations in diabetes. *Mol Metab*. 52:101280.
- 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 MS-MLPA Probemix ME033 TNDM

- Fu JL et al. (2019). Relapsed 6q24-related transient neonatal diabetes mellitus successfully treated with sulfonylurea. *Chin Med J*. 132(7), 846.
- Mustafa M et al. (2021). Transient Neonatal Diabetes Mellitus with the Rare Association of Nonsuppurative Sialadenitis and Genetic Defects in 6q24. *Case Reports in Pediatrics*. 2021:5901898.

ME033 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-04 – 05 December 2023 (04M) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Exon numbering of the *NMBR* and *PLAGL1* genes has been changed. - Ligation sites of the probes targeting the *ZFP57*, *NMBR, HIVEP2*, *PLAGL1, HYMAI, INS and KCNJ11* genes updated according to new version of the NM_ reference sequences. Version A1-03 — 24 March 2020 (02M) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Exon numbering of the *ZFP57* gene has been changed. - Ligation sites of the probes targeting the *ZFP57, HIVEP2, PEX3, ZC2HC1B*, *PLAGL1,* and *SASH1* genes updated according to new version of the NM_ reference sequences. - Contact email addresses and website URL updated. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. Version A1-02 – 06 September 2019 (01M) - Advice on not spinning down your MLPA reactions in between the ligation and PCR reaction was added to the warning about non-specific peaks on page 1. - Catalogue number SALSA HhaI adjusted. Version A1-01 – 05 December 2018 (01M) - Product description restructured and adapted to a new template. - Information for the *PLAGL1* probes adjusted in Table 1 and on page 5 - Warning on non-specific peaks included on page 1.

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