Product Description SALSA® MLPA® Probemix P241-E1 MODY Mix 1

To be used with the MLPA General Protocol.

Version E1. As compared to version D2, promoter and exon 1 probes for *HNF4A* (NM_175914.4) have been added; four *HNF4A* probes, two *GCK* probes, one *HNF1B* probe, and two reference probes have been replaced; and several probes have a small change in length. For complete product history see page 10.

Catalogue numbers:
- **P241-025R:** SALSA MLPA probemix P241 MODY Mix 1, 25 reactions.
- **P241-050R:** SALSA MLPA probemix P241 MODY Mix 1, 50 reactions.
- **P241-100R:** SALSA MLPA probemix P241 MODY Mix 1, 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.

Intended use: The SALSA MLPA probemix P241 MODY Mix 1 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *HNF4A*, *GCK*, *HNF1A*, and *HNF1B* genes in order to confirm a potential cause and clinical diagnosis for Maturity-Onset Diabetes of the Young (MODY) type 1, 2, 3, and 5, respectively. Furthermore, it is intended for the detection of deletions in the human *HNF1B* gene in order to confirm a potential cause and clinical diagnosis for Renal Cysts and Diabetes Syndrome (RCAD).

This product can also be used for molecular genetic testing of at-risk family members/individuals. It is intended for use with human DNA extracted from peripheral blood. Deletions or duplications obtained with the P241 MODY probemix must be verified by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in the aforementioned genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of the aforementioned genes. This assay is not intended to be used as standalone assay for clinical decisions. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Maturity-Onset Diabetes of the Young (MODY) is a distinct form of non-insulin-dependent diabetes mellitus (NDDM, also known as type II diabetes). MODY has a dominant autosomal inheritance and generally develops in individuals under the age of 25 years. Of all diabetes patients, 1-5% suffer from MODY. As described below, 11 forms of MODY are identified. Each form is associated with one gene. MODY 1, 2 and 3 account for approximately 70% of the MODY cases. Pathogenic mutations in additional genes, for example *ABCC8* and *KCNJ11*, have also been described. These additional subtypes together account for approximately 1% of MODY. More information is available at http://www.nature.com/ejhg/journal/v22/n9/full/ejhg201414a.html.

Renal Cysts and Diabetes Syndrome (RCAD) is an autosomal dominant disorder characterised by diabetes and nondiabetic renal disease resulting from abnormal renal development. Whole *HNF1B* gene deletions form a high proportion of RCAD cases. More information is available at https://omim.org/entry/137920.

- MODY 1 is a result of defects in the hepatocyte nuclear factor-4-alpha (*HNF4A*) gene. The protein encoded by this gene regulates the expression of *HNF1A*. Probes for *HNF4A* are included in this P241 probemix.
- MODY 2 is caused by mutations in the glucokinase gene (*GCK*). Probes for *GCK* are included in this P241 probemix.
- MODY 3 is caused by defects in the HNF1 homeobox A gene (*HNF1A*). Probes for *HNF1A* are included in this P241 probemix.
- MODY 4 has been linked to defects in the pancreas/duodenum homeobox protein 1 gene (*PDX1*). Probes for *PDX1* are included in the P357 probemix.
- MODY 5 has been associated with the HNF1 homeobox B gene (*HNF1B*). The gene is also associated with RCAD. Probes for *HNF1B* are included in this P241 probemix and in the P357 probemix.
- MODY 6 has been linked to defects in the *NEUROD1* gene. Probes for *NEUROD1* are included in the P357 probemix.
- MODY 7 is caused by mutations in the krüppel-like factor 11 gene (*KLF11*) on chromosome 2p25. Probes for *KLF11* are included in the P357 probemix.
- MODY 8 has been associated with defects in carboxyl-ester lipase gene (*CEL*). Probes for *CEL* are included in the P357 probemix.
- MODY 9 is caused by defects in the paired box 4 gene (*PAX4*). Probes for *PAX4* are included in the P357 probemix.
- MODY 10 has been linked to mutations in the insulin gene (*INS*). Probes for *INS* are included in the P357 probemix.
- MODY 11 has been linked to defects in the B lymphoid tyrosine kinase gene (*BLK*). No probes for *BLK* have been included in probemix P241 or probemix P357.

Gene structure: The *GCK* gene, located on chromosome 7p13, spans ~45 kb of genomic DNA and contains 11 exons. The LRG sequence is LRG_1074 sequence (on 04/2018, this sequence was pending approval) and is identical to the NCBI NG_008847.2 sequence.
The *HNF1A* gene on chromosome 12q24 spans ~24 kb of genomic DNA and comprises 10 exons. The LRG sequence is LRG_522 and is identical to the NCBI NG_011731.2 sequence.
The *HNF1B* gene, located on chromosome 17q12, spans ~59 kb of genomic DNA and contains 9 exons. This gene is also associated with RCAD, in which whole gene deletions account for approximately 50% of cases.
The NCBI NG sequence for the *HNF1B* gene is NG_013019.2. No LRG sequence is available.
The *HNF4A* gene spans ~77 kb of genomic DNA on chromosome 20q13 and comprises 12 exons. The LRG sequence is LRG_483 and is identical to the NCBI NG_009818.1 sequence.
The LRG sequences are available at http://www.lrg-sequence.org/.

Transcript variants:

- The NM_000162.4 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_000162.4, represents transcript variant 1 of the *GCK* gene. The ATG translation start site is located in exon 1 (487-489) and the stop codon is located in exon 11 (1882-1884). This pancreatic tissue-specific transcript lacks exon 2.
- The NM_000545.6 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_000545.6, represents transcript variant 2 of the *HNF1A* gene. The ATG translation start site is located in exon 1 (202-204) and the stop codon is located in exon 10 (2095-2097).
- The NM_000458.3 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_000458.3, represents transcript variant 1 of the *HNF1B* gene. This sequence is the reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 1 (195-197) and the stop codon is located in exon 9 (1866-1868).
- The NM_175914.4 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_175914.4, represents transcript variant 5 of the *HNF4A* gene. This sequence is a reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 1 (5-7) and the stop codon is located in exon 12 (1361-1363). This pancreatic tissue-specific transcript lacks exon 2 and exon 3. The NM_000457.4 sequence, also a reference standard in the NCBI RefSeqGene project, represents transcript variant 2 and lacks exon 1 and exon 2.

Exon numbering: The exon numbering used in this P241-E1 MODY Mix 1 product description for *HNF1A* is the exon numbering from the RefSeq transcript NM_000545.6.
For *HNF1B* the exon numbering used is from the RefSeq transcript NM_000458.3.
For *HNF4A* the exon numbering used is from the RefSeq transcript NM_175914.4.
The *GCK* exon numbering has changed. From description version 21 onwards, we have adopted the NCBI exon numbering that is present in the LRG_1074 sequence for this gene. The exon numbering used in previous versions of this product description can be found between brackets in Table 2.
The exon numbering, LRG and NM sequences are from 04/2018, but can be changed (by NCBI) after the release of the product description.

Probemix content: The P241-E1 MODY Mix 1 probemix contains 52 MLPA probes with amplification products between 130 and 500 nt. It contains probes for the *HNF4A*, *GCK*, *HNF1A*, and *HNF1B* genes and is therefore specific for MODY 1, 2, 3, and 5. For the *HNF4A* gene, 12 probes are included, furthermore 11 for the *GCK* gene, 11 for the *HNF1A* gene, and 10 for the *HNF1B* gene. In addition, eight reference probes are included in this probemix. 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, 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 and 96 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 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 MODY and RCAD. 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.

Performance characteristics: The expected number of deletions/duplications in *GCK*, *HNF1A*, *HNF1B,* and *HNF4A* which can be detected with this MLPA probemix is 1-3% of all mutations in most populations. The number of deletions in *HNF1B* which can be detected with this MLPA probemix is expected to be approximately 25% of RCAD cases in most patient populations. Analytical performance for the detection of deletions/duplications in the *GCK*, *HNF1A*, *HNF1B,* and *HNF4A* genes is very high and can be considered >99% (based on a 2008-2015 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for the *GCK*, *HNF1A*, *HNF1B,* and *HNF4A* specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). Copy numbers of 4 (heterozygous triplication/homozygous duplication) or 0 (homozygous deletion) may occur, but are extremely rare.
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 or in or near the *GCK*, *HNF1A*, *HNF1B*, and *HNF4A* genes. 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 *GCK*, *HNF1A*, *HNF1B*, and *HNF4A* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P241 MODY Mix 1.
- 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 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.

Mutation databases: DMuDB (https://secure.dmudb.net/ngrl-rep/Home.do) and LOVD (http://grenada.lumc.nl/LOVD2/diabetes/home.php and https://databases.lovd.nl/shared/genes/HNF1B). We strongly encourage users to deposit positive results in the above mentioned database. 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 *HNF1A* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P241-E1 MODY Mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a]				
		Reference	GCK	HNF1A	HNF1B	HNF4A
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 00797-L13645	5q31				
136	**HNF1B probe** 09858-L21367				**Exon 7**	
142	**GCK probe** 07721-L07431		**Exon 3**			
147	**HNF1A probe** 07710-L07442			**Exon 2**		
154	**HNF4A probe** 07734-L07424					**Exon 8**
160 «	**HNF1B probe** 07699-L07458				**Exon 2**	
166	**GCK probe** 07724-L07434		**Exon 6**			
172	**HNF1A probe** 07718-L07450			**Exon 10**		
178 *	**GCK probe** 21115-L29351		**Exon 11**			
184	**HNF1A probe** 07711-L07443			**Exon 3**		
190 *	Reference probe 20749-L28650	1q24				
196	**HNF1A probe** 07715-L07447			**Exon 7**		
202	**HNF1B probe** 08298-L09334				**Exon 8**	
208 *	**HNF4A probe** 21116-L29352					**Exon 9**
214 *	**HNF4A probe** 21117-L29353					**Exon 6**
220	**GCK probe** 07720-L07430		**Exon 1**			
226	**HNF1A probe** 07708-L07440			**Exon 1**		
232	**GCK probe** 07728-L07438		**Exon 10**			
238	**HNF1B probe** 16906-L19835				**Exon 5**	
244	Reference probe 13389-L14846	**6q12**				
251	**HNF1A probe** 07717-L07449			**Exon 9**		
259 ¥	**HNF4A probe** 21256-L07421					**Exon 4**
265	**GCK probe 07723-L21369**		**Exon 5**			
274	Reference probe 14110-L21370	**8p21**				
280	**HNF1A probe** 16907-L21371			**Exon 4**		
286	**HNF4A probe** 07737-L21372					**Exon 11**
295 ¥	**GCK probe** 07726-L29804		**Exon 8**			
301	**HNF1B probe** 16908-L19837				**Exon 4**	
310	Reference probe 14480-L16200	**4q12**				
319 *	**HNF4A probe** 21258-L29621					**Exon 12**
326	**HNF1A probe** 16752-L20209			**Exon 1**		
337	**GCK probe** 07722-L07432		**Exon 4**			
346	**HNF1A probe** 09856-L30357			**Exon 8**		
355	**HNF4A probe** 07736-L07426					**Exon 10**
364	**HNF1B probe** 07701-L29620				**Exon 4**	
372 *	Reference probe 08893-L23475	**14q24**				
379 *	**GCK probe** 21253-L29622		**Exon 9**			
386	**HNF1B probe** 16909-L21376				**Exon 3**	
393	**HNF4A probe** 07732-L21377					**Exon 5**
400 * ๑	**HNF4A probe** 21120-L29356					**Exon 3**
409	**HNF4A probe** 09999-L21378					**Exon 7**
418	**HNF1A probe** 07713-L07445			**Exon 5**		
427 ¥	**GCK probe** 07719-L29801		**Exon 1**			
433 *	**HNF1B probe** 21371-L29819				**Exon 9**	
445	Reference probe 14670-L16322	**19q13**				
454	**GCK probe** 07725-L07435		**Exon 7**			
462 *	**HNF4A probe** 21121-L29357					**Exon 1**
468	**HNF1B probe** 07704-L29802				**Exon 6**	
475 ¥	**HNF1A probe** 16913-L29803			**Exon 6**		
484 «	**HNF1B probe** 16912-L18619				**Exon 1**	
495 *	**HNF4A probe** 20962-L29109					**Exon 1**
500 ¥	Reference probe 12462-L19605	**22q12**				

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

* New in version E1 (from lot E1-0717 onwards).

¥ Changed in version E1 (from lot E1-0717 onwards). Change in length, no change in sequence detected.

« 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.
◎ The significance of exon 3 deletions/duplications is not clear as this exon is not present in transcript variant 5 (NM_175914.4).

Table 2. P241 probes arranged according to chromosomal location

Table 2a. *GCK* gene

Length (nt)	SALSA MLPA probe	GCK exon[a]	Ligation site[b] NM_000162.4	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*487-489 (ex 1)*		
427	07719-L29801	Exon 1	70-71 (5' UTR)	GCTAGGATGTGA-GAGACACAGTCA	0.4 kb
220	07720-L07430	Exon 1	508-509	ACAGAGCCAGGA-TGGAGGCCGCCA	35.5 kb
142	07721-L07431	Exon 3 (2)	600-601	AGACGGATGCAG-AAGGAGATGGAC	1.0 kb
337	07722-L07432	Exon 4 (3)	733-734	TGGGTGGCACTA-ACTTCAGGGTGA	1.4 kb
265	07723-L21369	Exon 5 (4)	905-906	GCATCAGATGAA-ACACAAGAAGCT	1.0 kb
166	07724-L07434	Exon 6 (5)	1024-1025	CAGAAGGGAACA-ATGTCGTGGGGC	0.2 kb
454	07725-L07435	Exon 7 (6)	1135-1136	GCTACTACGAAG-ACCATCAGTGCG	2.1 kb
295	07726-L29804	Exon 8 (7)	1304-1303 reverse	CCAGGCGGTCAT-ACTCCAGCAGGA	1.2 kb
379	21253-L29622	Exon 9 (8)	1479-1478 reverse	ACGAAGCGCGTC-TCGAAGGCTCCG	0.8 kb
232	07728-L07438	Exon 10 (9)	1541-1542	CTACAACATCCT-GAGCACGCTGGG	0.4 kb
178	21115-L29351	Exon 11 (10)	2 nt before exon 11 reverse	CTCCTTGAAGCT-GGGCAGAAGAGA	
		stop codon	*1882-1884 (ex 11)*		

(a) See above section on exon numbering for more information.
(b) Ligation sites of the P241 MODY Mix 1 MLPA probes are indicated according to RefSeq sequence NM_000162.4, containing 10 exons (1, 3-11).
(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 2b. *HNF1A* gene

Length (nt)	SALSA MLPA probe	HFN1A exon[a]	Ligation site[b] NM_000545.6	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*202-204 (ex 1)*		
226	07708-L07440	Exon 1	210-211	GCCATGGTTTCT-AAACTGAGCCAG	0.5 kb
326	16752-L20209	Exon 1	169 nt after exon 1	CTTGGAGGTTTG-AGCCTCCAGCCC	9.7 kb
147	07710-L07442	Exon 2	639-640	CACCTGTCCCAA-CACCTCAACAAG	4.6 kb
184	07711-L07443	Exon 3	782-783	AGGTGATGAGCT-ACCAACCAAGAA	0.7 kb
280	16907-L21371	Exon 4	1023-1024	CGGCGCAAAGAA-GAAGCCTTCCGG	2.0 kb
418	07713-L07445	Exon 5	1197-1198	GAGACTGCAGAA-GTACCCTCAAGC	0.3 kb
475	16913-L29803	Exon 6	1374-1375	AGCTTGGAGCAG-ACATCCCCAGGC	1.0 kb
196	07715-L07447	Exon 7	1631-1630 reverse	GCACAGGTGGCA-TGAGCGGCTGCT	1.7 kb
346	09856-L30357	Exon 8	1717-1716 reverse	CACCTCGGGCTT-GTGGCTGTAGAG	0.2 kb
251	07717-L07449	Exon 9	1836-1835 reverse	GCCTCAGTGTCT-GAGGTGAAGACC	1.6 kb
172	07718-L07450	Exon 10	1985-1986	CTCCAGCAGCCT-GGTGCTGTACCA	
		stop codon	*2095-2097 (ex 10)*		

(a) See above section on exon numbering for more information.
(b) Ligation sites of the P241 MODY Mix 1 MLPA probes are indicated according to RefSeq sequence NM_000545.6, containing 10 exons.
(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 2c. *HNF1B* gene

Length (nt)	SALSA MLPA probe	HFN1B exon[a]	Ligation site[b] NM_000458.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*195-197 (ex 1)*		
484 «	16912-L18619	Exon 1	199-200	TTGGAAAATGGT-GTCCAAGCTCAC	5.3 kb
160 «	07699-L07458	Exon 2	591-592	TGCAGCAACACA-ACATCCCCCAGA	5.9 kb
386	16909-L21376	Exon 3	876-877	CTGAGCCCACCA-ACAAGAAGATGC	1.9 kb
364 #	07701-L29620	Exon 4	1070-1069 reverse	ACACGGACCTCA-GTGACCAAGTTG	0.2 kb
301	16908-L19837	Exon 4	1231-1230 reverse	TACCTGACAGCT-TGTTTGGAGGAG	21.1 kb
238	16906-L19835	Exon 5	20 nt after exon 5 reverse	CTCCAGAGCGAC-AATGGCCCAGGT	5.5 kb
468	07704-L29802	Exon 6	1418-1419	GTCTCAGGAGGA-GGTTTGCCCCCA	4.0 kb
136	09858-L21367	Exon 7	1653-1652 reverse	GCTCTGCTGCAT-GAGGGGCTGCTG	2.0 kb
202	08298-L09334	Exon 8	1817-1818	AGCAGCATCAGT-ACACTCACCAAC	11.7 kb
433	21371-L29819	Exon 9	1871-1872	GCCTGGTGATGC-CCACACACCACT	
		stop codon	*1866-1868 (ex 9)*		

(a) See above section on exon numbering for more information.
(b) Ligation sites of the P241 MODY Mix 1 MLPA probes are indicated according to RefSeq sequence NM_000458.3, containing 9 exons.
(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

« Probe located within, or near a CpG island. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of CG rich regions.
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.

Table 2d. *HNF4A* gene

Length (nt)	SALSA MLPA probe	HFN4A exon[a]	Ligation site[b] NM_175914.4	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*5-7 (ex 1)*		
462 +	21121-L29357	Exon 1	157 before exon 1 reverse	ACCCAAGGTGGG-TGGATACGTTAA	0.2 kb
495	20962-L29109	Exon 1	41-40 reverse	GTAAGAACTCTC-CACTGGAGCCCC	45.6 kb
400 ๑	21120-L29356	Exon 3	NM_000457.4; 208-209	AATTTGAGAATG-TGCAGGTGTTGA	4.6 kb
259	21256-L07421	Exon 4	77-78	CAGAAGGCACCA-ACCTCAACGCGC	1.4 kb
393	07732-L21377	Exon 5	292-293	TGCAGGCTCAAG-AAATGCTTCCGG	6.3 kb
214	21117-L29353	Exon 6	369-370	AAGGTCAAGCTA-TGAGGACAGCAG	0.9 kb
409	09999-L21378	Exon 7	519-520	CATGAAGGAGCA-GCTGCTGGTTCT	3.8 kb
154	07734-L07424	Exon 8	5 nt before exon 8	TTCCTTCTCTCT-TTCAGGTGGCCC	1.4 kb
208	21116-L29352	Exon 9	820-819 reverse	CCTGGGTCAAAG-AAGATGATGGCT	4.4 kb
355	07736-L07426	Exon 10	1007-1008	AGCAGATCCAGT-TCATCAAGCTCT	4.3 kb
286	07737-L21372	Exon 11	19 nt after exon 11	AACTCTGGGATT-TTACCTTGCAAA	1.1 kb
319	21258-L29621	Exon 12	1346-1347	CGACCATCACCA-AGCAGGAAGTTA	0.2 kb
		stop codon	*1361-1363 (ex 12)*		

(a) See above section on exon numbering for more information.
(b) Ligation sites of the P241 MODY Mix 1 MLPA probes are indicated according to RefSeq sequence NM_175914.4, containing 10 exons (1, 4-12). Only the HNF4A exon 3 probe is indicated according to RefSeq sequence NM_000457.4, containing 10 exons (3-12).
(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

๑ The significance of exon 3 deletions is not clear as this exon is not present in transcript variant 5 (NM_175914.4).
+ This probe binds upstream of the 5' UTR of *HNF4A*.

Related SALSA MLPA probemixes

P117 ABCC8:	Contains probes for the *ABCC8* gene, involved in familial hyperinsulinemic hypoglycemia 1.
P297 Microdeletion syndromes-2:	Contains probes for the *HNF1B* gene and other genes involved in several microdeletion syndromes.
P357 MODY mix 2:	Contains probes for *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, and *INS* genes, involved in MODY 4 to 10.
ME033 TNDM:	Contains probes for the *INS* gene and other genes related to transient neonatal diabetes mellitus.

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P241 Product history	
Version	*Modification*
E1	Promoter and exon 1 probes for *HNF4A* (NM_175914.4) have been added; four *HNF4A* probes, two *GCK* probes, one *HNF1B* probe, and two reference probes have been replaced; and several probes have a small change in length.
D2	One reference probe has been replaced.
D1	Eight reference probes have been included; two *HNF1A* and four *HNF1B* probes have been replaced; the 88 and 96 nt control fragments have been replaced.
C2	Restricted release.
C1	Restricted release.
B1	One probe for the *HNF1A* gene has been added.
A1	First release.

Implemented changes in the product description

Version E1-05 – 15 June 2020 (04)
- Israel added as country with IVD status.

Version E1-04 – 15 February 2019 (04)
- A remark below Table 2d has been added mentioning that probe *HNF4A* exon 1 binds upstream of the 5' UTR.
- Reference added.
- Various minor textual changes.

Version E1-03 – 20 December 2018 (04)
- Regulatory status section updated to also include Morocco.

Version E1-02 – 04 July 2018 (04)
- Warning added to Table 2c for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Information about Gene structure, Transcript variants, Exon numbering, Positive control DNA samples and NF1 mutation database were adjusted.
- Information about RCAD added to the reference samples section.
- Product description adapted to a new template.
- Minor textual and layout changes.

Version E1-01 – 30 November 2017 (03)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Information about RCAD added.
- Remark about salt-sensitivity added to Table 1 and Table 2; 484 nt probe 16912-L18619 and 160 nt probe 07699-L07458.
- Various minor textual changes.

Version 21 – 11 April 2017 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 2, new pictures included).
- Exon numbering for the GCK gene has changed in Table 1 and 2.
- Various minor textual and layout changes.

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