Product Description

SALSA® MLPA® Probemix P241-E1 MODY Mix 1

To be used with the MLPA General Protocol.

Version E1
For complete product history see page 11.

Catalogue numbers:

- **P241-025R:** SALSA MLPA Probemix P241 MODY Mix1, 25 reactions.
- **P241-050R:** SALSA MLPA Probemix P241 MODY Mix1, 50 reactions.
- **P241-100R:** SALSA MLPA Probemix P241 MODY Mix1, 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.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 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.

Intended purpose
The SALSA MLPA Probemix P241 MODY Mix 1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *HNF4A, GCK, HNF1A*, and *HNF1B* genes in order to confirm a potential cause for and clinical diagnosis of Maturity-Onset Diabetes of the Young (MODY) type 1, 2, 3, and 5, respectively, and for molecular genetic testing of at-risk family members. It is intended for use with genomic DNA isolated from human peripheral whole blood specimens.

Copy number variations (CNVs) detected with P241 MODY Mix 1 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *HNF4A, GCK* and *HNF1A* genes, and some defects in the *HNF1B* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

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, 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, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
Maturity-onset diabetes of the young (MODY) is a group of inherited disorders of non-autoimmune diabetes mellitus which usually present in adolescence or young adulthood. MODY presents genetic, metabolic and clinical heterogeneity, however most cases share characteristics like β-cell dysfunction with an autosomal

dominant inheritance and early-onset (typically <35 years) of hyperglycaemia that is not insulin-dependent at point of diagnosis. Other characteristics include extrapancreatic features and absence of pancreatic autoimmunity markers. MODY is thought to account for at least 1-3% of all diabetes.
More information is available at https://www.ncbi.nlm.nih.gov/books/NBK500456/ and https://www.nature.com/articles/ejhg201414.

To date it has been proposed that pathogenic variants in at least 14 genes cause MODY. Each subtype is linked to a different gene. A portion of MODY may be caused by pathogenic variants in yet-to-be-identified genes or complex molecular alterations in the known MODY-related genes that were not detected by previous genetic testing methods. The four most common genetic causes of MODY are pathogenic variants in *GCK* (MODY 2) and *HNF1A* (MODY 3), each accounting for 30%-60% of all MODY, and *HNF4A* (MODY 1) and *HNF1B* (MODY 5 or renal cysts and diabetes (RCAD) syndrome), together accounting for about 10% of all MODY. MODY 5, also known as RCAD syndrome, is characterised by diabetes and nondiabetic renal disease resulting from abnormal renal development. Whole *HNF1B* gene deletions form a high proportion of RCAD cases. Other genes involved in MODY are listed in the table below.

MODY subtype	Gene	Description
MODY 1	*HNF4A*	The protein encoded by this gene regulates the expression of *HNF1A*. Pathogenic variants lead to β-cell dysfunction (mainly insulin secretory defect). Probes for *HNF4A* are included in this P241 probemix.
MODY 2	*GCK*	Pathogenic variants lead to β-cell dysfunction (glucose-sensing defect). Probes for *GCK* are included in this P241 probemix.
MODY 3	*HNF1A*	Pathogenic variants lead to β-cell dysfunction (mainly insulin secretory defect). Probes for *HNF1A* are included in this P241 probemix.
MODY 4	*PDX1*	Pathogenic variants lead to β-cell dysfunction. Probes for *PDX1* are included in the P357 probemix.
MODY 5 (RCAD)	*HNF1B*	Pathogenic variants lead to β-cell dysfunction. Probes for *HNF1B* are included in this P241 probemix and in the P357 probemix.
MODY 6	*NEUROD1*	Pathogenic variants lead to β-cell dysfunction. Probes for *NEUROD1* are included in the P357 probemix.
MODY 7	*KLF11*	Pathogenic variants lead to decreased glucose sensitivity of β-cells. Probes for *KLF11* are included in the P357 probemix.
MODY 8	*CEL*	Pathogenic variants lead to pancreatic endocrine and exocrine dysfunction. Probes for *CEL* are included in the P357 probemix.
MODY 9	*PAX4*	Pathogenic variants lead to β-cell dysfunction. Probes for *PAX4* are included in the P357 probemix.
MODY 10	*INS*	Pathogenic variants lead to β-cell dysfunction. Probes for *INS* are included in the P357 probemix.
MODY 11	*BLK*	Pathogenic variants lead to an insulin secretion defect.
MODY 12	*ABCC8*	Pathogenic variants lead to ATP-sensitive potassium channel dysfunction. Probes for *ABCC8* are included in the P117 probemix.
MODY 13	*KCNJ11*	Pathogenic variants lead to ATP-sensitive potassium channel dysfunction. Probes for *KCNJ11* are included in the ME033 probemix.
MODY 14	*APPL1*	Pathogenic variants lead to an insulin secretion defect.

Gene structure
The *HNF4A* gene spans 77 kilobases (kb) on chromosome 20q13 and contains 12 exons. The *HNF4A* LRG_483 is identical to GenBank NG_009818.1. The *GCK* gene spans 45 kb on chromosome 7p13 and contains 11 exons. The *GCK* LRG_1074 is identical to GenBank NG_008847.2. The *HNF1A* gene spans 24 kb on chromosome 12q24 and contains 10 exons. The *HNF1A* LRG_522 is identical to GenBank NG_011731.2. The *HNF1B* gene spans 59 kb on chromosome 17q12 and contains 9 exons. The NCBI NG sequence for the *HNF1B* gene is NG_013019.2. No LRG sequence is available.
The LRG sequences for *HNF4A*, *GCK* and *HNF1A* are available at www.lrg-sequence.org.

Transcript variants

For *HNF4A,* multiple transcript variants have been described. Transcript variant 5 encodes isoform 5 and is expressed specifically in pancreatic islet beta cells (NM_175914.5; 4558 nt; coding sequence 5-1363; https://www.ncbi.nlm.nih.gov/gene/3172). This sequence is a reference standard in the NCBI RefSeq project. Transcript variant 5 lacks exon 2 and 3. The resulting shorter isoform 5 has a distinct N-terminus compared to isoform 2. *HNF4A* transcript variant 2 encodes the longest isoform 2 and is also a reference standard in the NCBI RefSeq project (NM_000457.6; 6490 nt; coding sequence 135-1559). This variant lacks exon 1 and 2.

For *GCK,* multiple transcript variants have been described. Transcript variant 1 encodes isoform 1 and is expressed specifically in pancreatic islet beta cells (NM_000162.5; 2745 nt; coding sequence 487-1884; http://www.ncbi.nlm.nih.gov/gene/2645). This sequence is a reference standard in the NCBI RefSeq project. This variant lacks exon 2. Isoform 1 has a distinct N-terminus; the remainder of the protein is identical to isoforms 2 and 3.

For *HNF1A,* two transcript variants have been described. Transcript variant 2 is the most predominant and encodes isoform 2 (NM_000545.8; 3442 nt; coding sequence 227-2122; https://www.ncbi.nlm.nih.gov/gene/6927). This sequence is a reference standard in the NCBI RefSeq project.

For *HNF1B,* multiple transcript variants have been described. Transcript variant 1 represents the longer transcript and encodes the longer isoform 1 (NM_000458.4; 2790 nt; coding sequence 176-1849; https://www.ncbi.nlm.nih.gov/gene/6928). This sequence is a reference standard in the NCBI RefSeq project.

Exon numbering

The *HNF4A* exon numbering used in this P241-E1 MODY Mix 1 product description is the exon numbering from the LRG_483 sequence. For *GCK*, the exon numbering from the LRG_1074 sequence is used and for *HNF1A*, the exon numbering from the LRG_522 sequence is used. The *HNF1B* exon numbering used in this product description is the exon numbering from the NG_013019.2 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG or NG sequences. 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.

Probemix content

The SALSA MLPA Probemix P241-E1 MODY Mix 1 contains 52 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 12 probes for the *HNF4A* gene, 11 probes for the *GCK* gene, 11 probes for the *HNF1A* gene and 10 probes for the *HNF1B* gene. In addition, 8 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.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 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)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.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 human peripheral whole blood specimens, 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 different unrelated individuals who are from families without a history of MODY. More information regarding the selection and use of reference samples can be found in the 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. Sample ID numbers NA10925, NA07945, NA07081, NA10951 and NA20359 from the Coriell Institute have been tested with this P241-E1 probemix at MRC Holland and can be used as a positive control samples to detect the copy number alterations described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P241-E1	Expected copy number alteration
NA07945	Coriell Institute	20q13	*HNF4A*	Heterozygous deletion
NA10925	Coriell Institute	7p13	*GCK*	Heterozygous deletion
NA07081	Coriell Institute	7p13	*GCK*	Heterozygous duplication
NA10951	Coriell Institute	7p13	*GCK*	Heterozygous deletion
NA20359	Coriell Institute	17q12	*HNF1B*	Heterozygous duplication

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P241-E1 MODY Mix 1 probemix.

Performance characteristics
Partial and whole-gene deletions detectable by MLPA account for 1.9% of MODY 1, 1.8% of MODY 2, 1.2% of MODY 3, and 50% of MODY 5 (https://www.ncbi.nlm.nih.gov/books/NBK500456/). 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 2008-2021 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms 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 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 results

The expected results for the *HNF4A, GCK*, *HNF1A,* and *HNF1B* 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 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 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 (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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *HNF4A*, *GCK* and *HNF1A* genes, and some defects in the *HNF1B* gene are small (point) mutations, none of which will 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. 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.

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.

LOVD mutation database

We strongly encourage users to deposit positive results in the LOVD Database https://databases.lovd.nl/shared/genes/HNF4A; https://databases.lovd.nl/shared/genes/GCK; https://databases.lovd.nl/shared/genes/HNF1A; https://databases.lovd.nl/shared/genes/HNF1B. 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 *HNF1A* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

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

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*HNF4A*	*GCK*	*HNF1A*	*HNF1B*
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 00797-L13645	5q				
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	1q				
196	**HNF1A probe** 07715-L07447				**Exon 7**	
202	**HNF1B probe** 08298-L09334					**Exon 8**
208	**HNF4A probe** 21116-L29352		**Exon 9**			

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*HNF4A*	*GCK*	*HNF1A*	*HNF1B*
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	6q				
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	8p				
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	4q				
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	14q				
379	**GCK probe** 21253-L29622			**Exon 9**		
386	**HNF1B probe** 16909-L21376					**Exon 3**
393	**HNF4A probe** 07732-L21377		**Exon 5**			
400 ◎	**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	19q				
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	22q				

[a] See section Exon numbering on page 3 for more information.

« 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_175914.5).

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. P241-E1 probes arranged according to chromosomal location

Table 2a. *HNF4A* gene

Length (nt)	SALSA MLPA probe	*HNF4A* exon[a]	Ligation site NM_175914.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*107-109 (Exon 1)*		
462 +	21121-L29357	Exon 1	55 nt before exon 1 reverse	ACCCAAGGTGGG-TGGATACGTTAA	0.2 kb
495	20962-L29109	Exon 1	143-142 reverse	GTAAGAACTCTC-CACTGGAGCCCC	45.6 kb
400 ◎	21120-L29356	Exon 3	NM_000457.6; 225-226	AATTTGAGAATG-TGCAGGTGTTGA	4.6 kb
259	21256-L07421	Exon 4	179-180	CAGAAGGCACCA-ACCTCAACGCGC	1.4 kb
393	07732-L21377	Exon 5	394-395	TGCAGGCTCAAG-AAATGCTTCCGG	6.3 kb
214	21117-L29353	Exon 6	471-472	AAGGTCAAGCTA-TGAGGACAGCAG	0.9 kb
409	09999-L21378	Exon 7	621-622	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	922-921 reverse	CCTGGGTCAAAG-AAGATGATGGCT	4.4 kb
355	07736-L07426	Exon 10	1109-1110	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	1448-1449	CGACCATCACCA-AGCAGGAAGTTA	
		stop codon	*1463-1465 (Exon 12)*		

[a] See section Exon numbering on page 3 for more information.

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

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

+ This probe binds upstream of the 5' UTR of *HNF4A*.

Table 2b. *GCK* gene

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

[a] See section Exon numbering on page 3 for more information.

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Table 2c. *HNF1A* gene

Length (nt)	SALSA MLPA probe	*HNF1A* exon[a]	Ligation site NM_000545.8	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*227-229 (Exon 1)*		
226	07708-L07440	Exon 1	235-236	GCCATGGTTTCT-AAACTGAGCCAG	0.5 kb
326	16752-L20209	Exon 1	170 nt after exon 1	CTTGGAGGTTTG-AGCCTCCAGCCC	9.7 kb
147	07710-L07442	Exon 2	664-665	CACCTGTCCCAA-CACCTCAACAAG	4.6 kb
184	07711-L07443	Exon 3	807-808	AGGTGATGAGCT-ACCAACCAAGAA	0.7 kb
280	16907-L21371	Exon 4	1048-1049	CGGCGCAAAGAA-GAAGCCTTCCGG	2.0 kb
418	07713-L07445	Exon 5	1222-1223	GAGACTGCAGAA-GTACCCTCAAGC	0.3 kb
475	16913-L29803	Exon 6	1399-1400	AGCTTGGAGCAG-ACATCCCCAGGC	1.0 kb
196	07715-L07447	Exon 7	1656-1655 reverse	GCACAGGTGGCA-TGAGCGGCTGCT	1.7 kb
346	09856-L30357	Exon 8	1742-1741 reverse	CACCTCGGGCTT-GTGGCTGTAGAG	0.2 kb
251	07717-L07449	Exon 9	1861-1860 reverse	GCCTCAGTGTCT-GAGGTGAAGACC	1.6 kb
172	07718-L07450	Exon 10	2010-2011	CTCCAGCAGCCT-GGTGCTGTACCA	
		stop codon	*2120-2122 (Exon 10)*		

[a] See section Exon numbering on page 3 for more information.

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Table 2d. *HNF1B* gene

Length (nt)	SALSA MLPA probe	*HNF1B* exon[a]	Ligation site NM_000458.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*176-178 (Exon 1)*		
484 «	16912-L18619	Exon 1	180-181	TTGGAAAATGGT-GTCCAAGCTCAC	5.3 kb
160 «	07699-L07458	Exon 2	572-573	TGCAGCAACACA-ACATCCCCCAGA	5.9 kb
386	16909-L21376	Exon 3	857-858	CTGAGCCCACCA-ACAAGAAGATGC	1.9 kb
364 #	07701-L29620	Exon 4	1051-1050 reverse	ACACGGACCTCA-GTGACCAAGTTG	0.2 kb
301	16908-L19837	Exon 4	1212-1211 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	1399-1400	GTCTCAGGAGGA-GGTTTGCCCCCA	4.0 kb
136	09858-L21367	Exon 7	1634-1633 reverse	GCTCTGCTGCAT-GAGGGGCTGCTG	2.0 kb
202	08298-L09334	Exon 8	1798-1799	AGCAGCATCAGT-ACACTCACCAAC	11.7 kb
433	21371-L29819	Exon 9	1852-1853	GCCTGGTGATGC-CCACACACCACT	
		stop codon	*1847-1849 (Exon 9)*		

[a] See section Exon numbering on page 3 for more information.

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

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

This probe'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.

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.

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

P117 ABCC8	Contains probes for the *ABCC8* gene (MODY 12), involved in familial hyperinsulinemic hypoglycemia 1.
P297 Microdeletion Syndromes-2	Contains probes for the *HNF1B* gene (MODY 5) 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 (MODY 10), the *KCNJ11* gene (MODY 13) 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) 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	Three *HNF4A* probes, three *HNF1A* probes and two *HNF1B* probes have been replaced.
A1	First release.

Implemented changes in the product description
Version E1-07 – 04 December 2023 (04P) - Product is no longer registered for IVD use in Morocco. Version E1-06 – 01 July 2021 (04P) - Product description rewritten and adapted to a new template. - UK has been added to the list of countries in Europe that accept the CE mark. - Information about renal cysts and diabetes (RCAD) syndrome has been adapted, as it is now considered an alternative name for MODY 5. - Clinical background section updated with new information from the literature. - Ligation sites of the probes targeting the *HNF4A*, *GCK*, *HNF1A* and *HNF1B* genes updated according to new version of the NM_ reference sequence. - Three recent articles added to the list of Selected publications. 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.

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IVD	EUROPE* CE ISRAEL
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.