Product Description
SALSA® MLPA® Probemix P055-D1 PAH

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 9.

Catalogue numbers:
- **P055-025R:** SALSA MLPA Probemix P055 PAH, 25 reactions.
- **P055-050R:** SALSA MLPA Probemix P055 PAH, 50 reactions.
- **P055-100R:** SALSA MLPA Probemix P055 PAH, 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 P055 PAH 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 *PAH* gene in genomic DNA isolated from human peripheral whole blood specimens and DNA from cells collected with buccal swabs. P055 PAH is intended to confirm a potential cause for and clinical diagnosis of phenylalanine hydroxylase deficiency in patients diagnosed with phenylketonuria (PKU) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P055 PAH 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 *PAH* 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, parental evaluation, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

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

[1]Please note that this probemix is for 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
Phenylketonuria (PKU), as well as the less severe forms of the condition (sometimes called variant PKU or non-PKU hyperphenylalaninemia), is an autosomal recessive disease resulting in a metabolic defect characterised by hyperphenylalaninemia resulting from a deficiency of phenylalanine hydroxylase (98% of

cases) or impaired synthesis or recycling of tetrahydrobiopterin (2% of cases) (Scriver and Kaufman 2001). Untreated PKU can lead to microcephaly, epilepsy, severe mental retardation and behavioural problems. More information on PKU is available on http://www.ncbi.nlm.nih.gov/books/NBK1504/.

The majority of PKU cases are due to defects in the *PAH* gene. Among these defects are deletions and duplications of complete exons, which are usually missed by standard sequence analysis. The MLPA technique can detect most of these deletions and duplications and therefore complements sequence analysis of the *PAH* gene. The expected number of *PAH* chromosomal rearrangements that can be detected with this MLPA probemix is between 1 and 5% of all PKU cases in most populations (see below for publications on probemix P055 PAH).

Gene structure
The *PAH* gene spans ~121 kilobases (kb) on chromosome 12q23.2 and contains 13 exons. No LRG sequence for *PAH* is available at this moment. The GenBank chromosomal DNA sequence is NG_008690.2

Transcript variants
Two *PAH* transcript variants have been defined. Transcript variant 1 (NM_000277.3, 3759 nucleotides (nt), coding sequence 115-1473) is the reference standard in the NCBI RefSeqGene project www.ncbi.nlm.nih.gov/gene/5053.

Exon numbering
The *PAH* exon numbering used in this P055-D1 PAH product description is the exon numbering from the NG_008690.2 sequence. As changes to the databases can occur after release of this product description, the NG_ and NM_ sequences, and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P055-D1 PAH contains 38 MLPA probes with amplification products between 128 and 427 nt. This includes 22 probes for the *PAH* gene and one probe upstream and downstream of *PAH*. In addition, 14 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).

Several partially conserved and potential regulatory elements are located just upstream of *PAH* exon 1. These DNaseI hypersensitive sites have been described by Bristeau et al. (2001). Two probes are located in these upstream sequences (418 nt HSS3 and 154 nt HSS2).

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 blood or buccal swabs, 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 (variant) PKU. 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. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics
The expected percentage of *PAH* chromosomal rearrangements that can be detected with this MLPA probemix is between 1 and 5% of all PKU cases in most populations (see below for publications on probemix P055 PAH). The analytical sensitivity and specificity for the detection of deletions/duplications in the *PAH* gene is very high and can be considered >95%.

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 *PAH* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous duplication) and occasionally 4 (homozygous duplication or heterozygous triplication).

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 *PAH* gene. 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.
- 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.

P055 specific notes:

- PKU is caused by defects in the *PAH* gene and is an autosomal recessive disease. Inactivation of both copies of the *PAH* gene is expected to result in PKU. Inactivation of only a single copy of the *PAH* gene is typically seen in carriers.
- Deletion of one or more exons usually results in inactivation of that gene copy. Single exon deletions however have a considerable chance of being a false positive result. A 3.7 kb deletion in the 5'-flanking region of the *PAH* gene, including the DNaseI hypersensitive sites (HSS2 and HSS3 region, described by Bristeau et al. (2001)) has been found in a hyperphenylalaninemic patient (Chen et al. 2002).

- Duplication of an internal part of a gene usually results in a defective copy of that gene, as the duplicated sequence is typically located directly adjacent to the original sequence, resulting in a defective transcript. Duplication of the complete *PAH* gene is not expected to result in disease.
- Deletion or duplication of the flanking probes for *ASCL1* and *IGF1* are not expected to be the cause of PKU. These probes have only been included to delineate the extent of large deletions and duplications.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *PAH* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P055 PAH.
- 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.

***PAH* mutation database**

http://www.biopku.org/home/pah.asp**.** We strongly encourage users to deposit positive results in the Phenylalanine Hydroxylase Gene Locus-Specific 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 SNVs and unusual results (e.g., a duplication of *PAH* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P055-D1 PAH

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*PAH*
64-105	Control fragments – see table in probemix content section for more information		
128	Reference probe 00797-L00093	5q	
136	Reference probe 07292-L06929	6q	
142	**PAH** probe 02326-L01823		**Exon 7**
149 « ¬	**ASCL1** probe 02327-L01835		**Exon 2**
154	**HSS2** probe 12251-L14053		upstream
161	**PAH** probe 02328-L11413		**Exon 8**
168	**PAH** probe 16487-L23233		**Exon 1**
174	Reference probe 01823-L23229	16p	
180	**PAH** probe 16488-L23230		**Exon 2**
187	**PAH** probe 02331-L23231		**Exon 9**
194	Reference probe 05703-L06959	3q	
201 ±	**PAH** probe 16489-L18945		**Exon 3**
211	**PAH** probe 02333-L01826		**Exon 10**
220	Reference probe 01782-L01346	13q	
227	**PAH** probe 17737-L21083		**Exon 6**
235	**PAH** probe 02334-L23232		**Exon 4**
242	**PAH** probe 02335-L14055		**Exon 11**
247	Reference probe 07695-L07419	21q	
256	**PAH** probe 02336-L01821		**Exon 5**
265	**PAH** probe 02337-L02469		**Exon 12**
274	Reference probe 15473-L17313	1p	
283	**PAH** probe 16491-L18947		**Exon 6**
292	**PAH** probe 02339-L01829		**Exon 13**
300	Reference probe 01575-L01147	22q	
310 ¬	**IGF1** probe 02340-L01834		**Exon 2**
319	Reference probe 06440-L05966	3p	
337	**PAH** probe 12254-L14056		**Exon 1**
346	**PAH** probe 16492-L18948		**Exon 5**
352	**PAH** probe 12256-L14058		**Exon 7**
359 Ж	Reference probe 13731-SP0136-L15212	15q	
365 Ж ±	**PAH** probe 16493-SP0373-L18949		**Exon 3**
373	**PAH** probe 16494-L18950		**Exon 2**
382	Reference probe 13974-L15543	7q	
391	Reference probe 12522-L13572	18q	
400	**PAH** probe 12260-L14061		**Exon 4**
409	Reference probe 10063-L10487	8q	
418	**HSS3** probe 12261-L13203		upstream
427	Reference probe 05915-L17921	14q	

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

± SNP rs192439649 could influence the 365 nt probe signal and SNP rs542737289 could influence the 201 nt probe. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. *PAH* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_000277.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
149 « ¬	02327-L01835	*ASCL1* gene		ACCTGCATCTTT-AGTGCTTTCTTG	38.9 kb
418	12261-L13203	HSS3 region	3238 nt before exon 1	CAATGGTTGGGT-AATCTTCAACTT	2.0 kb
154	12251-L14053	HSS2 region	1280 nt before exon 1	GTGGTAGAACCA-AGAGTTAAACCA	1.0 kb
		PAH start codon	*115-117 (Exon 1)*		
337	12254-L14056	PAH exon 1	291 nt before exon 1	GGCTTAGTCCAA-TTGCAGAGAACT	0.4 kb
168	16487-L23233	PAH exon 1	72-73	CTGCCTGTACCT-GAGGCCCTAAAA	4.1 kb
373	16494-L18950	PAH exon 2	191 nt before exon 2	GTAGCATCATTG-ATCATTTAATTG	0.4 kb
180	16488-L23230	PAH exon 2	73 nt after exon 2	AGTTAGATGCAA-TGAAAAGAACAC	17.6 kb
365 Ж ±	16493-SP0373-L18949	PAH exon 3	235 and 201 nt before exon 3	ATTTTCATGTGA-34 nt spanning oligo-CCTGCCACTTAG	0.4 kb
201 ±	16489-L18945	PAH exon 3	33 nt after exon 3	CAACATAAGTAA-CTCCACACTGTC	17.2 kb
235	02334-L23232	PAH exon 4	484-485	GGTTCCCAAGAA-CCATTCAAGAGC	0.1 kb
400	12260-L14061	PAH exon 4	3 nt after exon 4	GACCACCCTGTG-AGTCCATGGCCC	10.7 kb
346	16492-L18948	PAH exon 5	104 nt before exon 5	CCAAGGGAAGGA-GACATGCACTGT	0.2 kb
256	02336-L01821	PAH exon 5	613-614	ACATTGCCTACA-ACTACCGCCAGT	11.2 kb
283	16491-L18947	PAH exon 6	106 nt before exon 6 reverse	TGAGCTGCCATC-ACTTGCTACAGT	0.1 kb
227	17737-L21083	PAH exon 6	665-664 reverse	CCCATGTTTTCT-TTTCTTCCTCCA	2.4 kb
142	02326-L01823	PAH exon 7	877-878	CTCGGGATTTCT-TGGGTGGCCTGG	0.1 kb
352	12256-L14058	PAH exon 7	37 nt after exon 7	TTGCCAGGCACA-ATGAGCGCCATC	1.1 kb
161	02328-L11413	PAH exon 8	986-987	GTTGGGACATGT-GCCCTTGTTTTC	4.8 kb
187	02331-L23231	PAH exon 9	1067-1066 reverse	CGAGCTTTTCAA-TGTATTCATCAG	2.5 kb
211	02333-L01826	PAH exon 10	1142-1143	CATAAAGGCATA-TGGTGCTGGGCT	0.6 kb
242	02335-L14055	PAH exon 11	1230-1231	CTGGAGAAGACA-GCCATCCAAAAT	3.3 kb
265	02337-L02469	PAH exon 12	1385-1386	GATTGAGGTCTT-GGACAATACCCA	1.3 kb
292	02339-L01829	PAH exon 13	1471-1472	AGAAAATAAAGT-AAAGCCATGGAC	363.4 kb
		PAH stop codon	*1471-1473 (Exon 13)*		
310 ¬	02340-L01834	*IGF1* gene		AGGTAGAAGAGA-TGCGAGGAGGAC	

[a] See section Exon numbering on page 2 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.

± SNP rs192439649 could influence the 365 nt probe signal and SNP rs542737289 could influence the 201 nt probe. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P076 ACADVL-SLC22A5 Contains probes for the *ACADVL* gene, involved in Very Long-Chain Acyl-coenzyme A Dehydrogenase (VLCAD) deficiency.

References

- Bristeau A et al. (2001). Conserved as well as divergent regulatory elements account for expression of the human and rodent phenylalanine hydroxylase genes. *Gene.* 274:283-291.
- Chen KJ et al. (2002). Identification and characterization of a novel liver-specific enhancer of the human phenylalanine hydroxylase gene. *Human genetics.* 110:235-243.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic acids research.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Human mutation.* 28:205.
- Scriver CR et al. (2001) Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly SW, Valle D, editors. The Metabolic and Molecular Bases of Inherited Disease. 8 ed. New York, NY: McGraw-Hill. pp. 1667-1724.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Analytical biochemistry.* 421:799-801.

Selected publications using SALSA MLPA Probemix P055 PAH

- Birk Moller L et al. (2007). Low proportion of whole exon deletions causing phenylketonuria in Denmark and Germany. *Human mutation.* 28:207.
- Cali F et al. (2010). Exon deletions of the phenylalanine hydroxylase gene in Italian hyperphenylalaninemics. *Experimental & molecular medicine.* 42:81-86.
- Desviat LR et al. (2006). Identification of exonic deletions in the PAH gene causing phenylketonuria by MLPA analysis. *Clinica chimica acta; international journal of clinical chemistry.* 373:164-167.
- Gemperle-Britschgi C et al. (2016). A novel common large genomic deletion and two new missense mutations identified in the Romanian phenylketonuria population. *Gene.* 576:182-188.
- Groselj U et al. (2012). Five novel mutations and two large deletions in a population analysis of the phenylalanine hydroxylase gene. *Molecular genetics and metabolism.* 106:142-148.
- Ji CY et al. (2011). [The research of combining high resolution melting with multiplex ligation-dependent probe amplification technology in the mutation scanning for PAH gene]. *Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese journal of medical genetics.* 28:649-653.
- Kozak L et al. (2006). Identification and characterization of large deletions in the phenylalanine hydroxylase (PAH) gene by MLPA: evidence for both homologous and non-homologous mechanisms of rearrangement. *Molecular genetics and metabolism.* 89:300-309.
- Lee YW et al. (2008). Mutation analysis of PAH gene and characterization of a recurrent deletion mutation in Korean patients with phenylketonuria. *Experimental & molecular medicine.* 40:533-540.
- Lu CX et al. (2011). [Mutation analysis of phenylalanine hydroxylase gene in 55 patients with phenylketonuria from Hebei province]. *Zhonghua yi xue za zhi.* 91:2971-2976.
- Maruo Y et al. (2015). A novel large deletion (exons 12, 13) and a missense mutation (p.G46R) in the PAH in a Japanese patient with phenylketonuria. *World journal of pediatrics : WJP.* 11:181-184.
- Okano Y et al. (2011). Molecular characterization of phenylketonuria and tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency in Japan. *Journal of human genetics.* 56:306-312.
- Polak E et al. (2013). Phenylalanine hydroxylase deficiency in the Slovak population: genotype-phenotype correlations and genotype-based predictions of BH4-responsiveness. *Gene.* 526:347-355.
- Tao Y et al. (2021). Spectrum of PAH gene mutations and genotype-phenotype correlation in patients with phenylalanine hydroxylase deficiency from Shanxi province. *Brain Dev.* 43:220-229.
- Yan YS et al. (2016). [Analysis of large deletion of phenylalanine hydroxylase gene in Chinese patients with phenylketonuria]. *Zhonghua yi xue za zhi.* 96:1097-1102.

P055 product history	
Version	*Modification*
D1	One PAH exon 6 probe added, one PAH exon 12 probe (wild-type sequence at R408W mutation) removed. One reference probe replaced.
C1	Seven PAH probes and eight reference probes replaced. New 88 and 96 nt control fragments (QDX2).
B1	An increase of PAH probes, from 13 to 22. New control fragments.
A1	First release.

Implemented changes in the product description

Version D1-06 – 25 October 2022 (04P)
- Morocco has been removed from the list of countries with IVD status.

Version D1-05 – 15 October 2021 (04P)
- Product description adapted to a new template.
- Various minor textual changes.
- Warning for SNP (201 nt probe) is added under table 1 and 2.
- Minor correction were made to the ligation sites of the 187 nt and 283 nt probes in Table 2.
- Selected publication section is updated.
- UK added to the list of countries in Europe that accept the CE mark.

Version D1-04 – 29 June 2020 (02P)
- Product description adapted to a new template.
- Israel added as a country with IVD status.
- Ligation sites of the probes targeting the *PAH* gene updated according to new version of the NM_ reference sequence.
- NG_ reference sequence has been updated.
- Remark added to *PAH* exon 3 365 nt probe regarding the potential influence of a SNP.

Version D1-03 – 17 July 2018 (04)
- Product description restructured and adapted to a new template.
- IVD use now includes Morocco.
- Information about new and changed probes in version D1 compared to C1 removed from table 1 and 2.

Version D1-02 – 04 April 2017 (03)
- Product description adapted to a new template.
- New publications added which used P055.

Version D1-01 - 18 January 2016 (02)
- Product description restructured and adapted to a new template.
- Selected publications list updated.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

IVD	EUROPE* CE 0344 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.