Product Description SALSA® MLPA® Probemix P351-C1 PKD1 and P352-D1 PKD1-PKD2

To be used with the MLPA General Protocol.

P351 Version C1.
P352 Version D1.
For complete product history see page 9.

Catalogue numbers:
- **P351-025R:** SALSA MLPA Probemix P351 PKD1, 25 reactions.
- **P351-050R:** SALSA MLPA Probemix P351 PKD1, 50 reactions.
- **P351-100R:** SALSA MLPA Probemix P351 PKD1, 100 reactions.

- **P352-025R:** SALSA MLPA Probemix P352 PKD1-PKD2, 25 reactions.
- **P352-050R:** SALSA MLPA Probemix P352 PKD1-PKD2, 50 reactions.
- **P352-100R:** SALSA MLPA Probemix P352 PKD1-PKD2, 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.

General Information: The SALSA MLPA Probemix P351 PKD1 and P352 PKD1-PKD2 is a **research use only (RUO)** assay for the detection of deletions or duplications in *PKD1* and *PKD2*, which are associated with Autosomal Dominant Polycystic Kidney Disease (ADPKD).

ADPKD is an autosomal disorder, which is characterized by bilateral renal cysts, liver cysts and intracranial aneurysms. Fifty percent of ADPKD patients develop end stage renal disease at the age of 60, where patients with a *PKD1* mutation show a more progressive renal phenotype compared to patients with *PKD2* mutations (Choi et al. 2014). Although all individuals with ADPKD develop cysts within the kidneys, there is substantial variability in severity of renal disease and other manifestations of the disease, even within the same family.
Approximately 85% of patients with ADPKD have pathogenic variants in *PKD1*, whereas approximately 15% have pathogenic variants in *PKD2*. DNA analysis of the *PKD1* gene is complicated due to the presence of several *PKD1* pseudogenes on 16p13.11 at 16 Mb from the p-telomere. The *PKD1* product, polycystin-1, is a membrane-associated protein, though its exact function is not exactly known. It is thought to play a role in the polycystin complex, by regulating the polycystin-2 calcium channel, which is encoded by *PKD2*. Both proteins are also present in primary cilia. Mutations in either *PKD1* or *PKD2* likely disrupt the protein interaction and alter signalling within the cell and in primary cilia, causing cysts. Around 2-4% of *PKD1/PKD2* mutations are single- and multi-exon deletions and/or duplications (Carrera et al. 2016, Choi et al. 2014, Obeidova et al. 2014).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1246/.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and Transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene

For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Probemix content: The SALSA MLPA Probemixes P351-C1 PKD1 and P352-D1 PKD1-PKD2 contain 27 and 13 probes for *PKD1*, respectively. Together, these probemixes cover 36 of the 46 exons of *PKD1*. There are two probes upstream of *PKD1*, 3 probes for exon 15 of *PKD1* and 3 probes for the flanking gene *TSC2*, located just downstream of *PKD1*. In addition, P352-D1 includes 17 probes covering all *PKD2* exons with the exception of exon 13. There are two probes present for exon 1, 2 and 6.

The P351-C1 probemix contains 41 MLPA probes with amplification products between 135 and 459 nt, which includes 11 reference probes detecting 11 different autosomal chromosomal locations. The P352-D1 probemix contains 41 MLPA probes with amplifications products between 136 and 471 nt, of which 11 are reference probes detecting 11 different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

These probemixes contain 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.mpla.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 or 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

Required specimens: Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the 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 polycystic kidney disease. 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.

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.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 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 (Consugar et al. 2008). 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. The complete *PKD1* gene is located within such a strong GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by 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 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.

Notes for P351 and P352
- No probes are present for exons 1, 2, 4, 8, 17, 24, 28, 32, 34 and 45. This is due to the existence of several *PKD1* pseudogenes that are almost identical to the actual gene. These are also present on chromosome 16. Probes detecting exon 3-31 of *PKD1* depend for their specificity on a single nucleotide difference between the *PKD1* gene and the pseudogenes. For these probes, an apparent duplication can be the result of a clinically non-significant one nucleotide sequence change in one of these pseudogenes.

Limitations of the procedure:
- In most populations, the major cause of genetic defects in the *PKD1* and *PKD2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemixes P351/P352 PKD1-PKD2.
- 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 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.

ADPKD mutation database: http://pkdb.mayo.edu/. We strongly encourage users to deposit positive results in the ADPKD mutation 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 *PKD1* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P351-C1 PKD1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*PKD1*
64-105	Control fragments – see table in probemix content section for more information		
135	Reference probe 00797-L26847	5q31	
141 «	**PKD1 probe** 10938-L24677		**Exon 3**
148 «	**PKD1 probe** 10960-L16105		**Exon 40**
154 «	**PKD1 probe** 10950-L24689		**Exon 20**
160 «	**PKD1 probe** 10961-L24698		**Exon 41**
166	Reference probe 02310-L24631	19p13	
172 «	**PKD1 probe** 10943-L24681		**Exon 11**
178 «	**PKD1 probe** 10958-L24696		**Exon 38**
184 «	**PKD1 probe** 10963-L24700		**Exon 43**
190	Reference probe 08528-L24624	9p24	
196 «	**PKD1 probe** 10946-L27456		**Exon 14**
202 «	**PKD1 probe** 10962-L24699		**Exon 42**
218	Reference probe 07634-L24625	10p14	
226 «	**PKD1 probe** 10952-L24690		**Exon 23**
232 «	**PKD1 probe** 14095-L24702		**Exon 46**
239 «	**PKD1 probe** 10947-L24687		**Exon 16**
244	Reference probe 07997-L24623	7q21	
251 «	**PKD1 probe** 14096-L24686		**Exon 15**
258 «	**PKD1 probe** 10956-L24694		**Exon 30**
266 «	**PKD1 probe** 10942-L27362		**Exon 10**
274 «	**PKD1 probe** 10954-L24692		**Exon 27**
281	Reference probe 08887-L24627	12q21	
290 «	**PKD1 probe** 10949-L24688		**Exon 18**
297 «	**PKD1 probe** 10940-L24678		**Exon 5**
307 «	**PKD1 probe** 10944-L24682		**Exon 12**
315	Reference probe 12443-L13444	22q12	
322 «	**PKD1 probe** 10955-L24693		**Exon 29**
335 «	**PKD1 probe** 10964-L27363		**Exon 44**
343 «	**PKD1 probe** 14097-L24685		**Exon 15**
353	Reference probe 07082-L24626	11p13	
361 «	**PKD1 probe** 19919-L24683		**Exon 13**
370 «	**TSC2 probe** 11935-L24630		TSC2 Exon 41
379 «	**PKD1 probe** 10959-L24697		**Exon 39**
387	Reference probe 07925-L21227	20p13	
394 «	**PKD1 probe** 10941-L24679		**Exon 9**
414 «	**PKD1 probe** 10953-L24691		**Exon 25**
424 «	**TSC2 probe** 01842-L24628		TSC2 Exon 36
431	Reference probe 15541-L25346	2q23	
442 «	**TSC2 probe** 01843-L24629		TSC2 Exon 38
450 «	**PKD1 probe** 19918-L24695		**Exon 35**
459	Reference probe 01799-L23610	13q14	

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

Table 1b. SALSA MLPA Probemix P352-D1 PKD1-PKD2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		
		Reference	*PKD1*	*PKD2*
64-105	Control fragments – see table in probemix content section for more information			
136	Reference probe 00797-L24120	5q31		
142 «	**PKD2 probe** 10995-L24648			**Exon 2**
148	**PKD2 probe** 11005-L24659			**Exon 12**
153 «	**PKD1 probe** 14100-L24666		**Exon 7**	
159 «	**PKD1 probe** 10992-L24674		**Exon 36**	
166	**PKD2 probe** 11004-L24658			**Exon 11**
172	Reference probe 03245-L24643	13q14		
178	**PKD2 probe** 11007-L24661			**Exon 14**
185 «	**PKD1 probe** 19917-L11651		**Exon 6**	
191 «	**PKD1 probe** 11011-L27419		**Exon 31**	
202	**PKD2 probe** 11000-L24654			**Exon 7**
214	Reference probe 18153-L22663	21q22		
220	**PKD2 probe** 10998-L24652			**Exon 5**
233	**PKD2 probe** 14739-L24653			**Exon 6**
240	Reference probe 09278-L24642	11q13		
248 «	**PKD1 probe** 10983-L24667		**Exon 15**	
257 «	**PKD1 probe** 14102-L24664		**Upstream**	
263	**PKD2 probe** 14738-L20398			**Exon 6**
270 «	**PKD1 probe** 10986-L24669		**Exon 21**	
280	Reference probe 10799-L24638	2q36		
291 «	**PKD1 probe** 10984-L24668		**Exon 19**	
297 «	**PKD2 probe** 10994-L24647			**Exon 1**
307	Reference probe 18034-L22721	17q11		
315 «	**PKD1 probe** 11009-L24671		**Exon 26**	
325	**PKD2 probe** 11002-L24656			**Exon 9**
334	Reference probe 01918-L24637	1q22		
342	**PKD2 probe** 10997-L24651			**Exon 4**
352	**PKD2 probe** 11003-L24657			**Exon 10**
362	Reference probe 06383-L24641	8p23		
371	**PKD2 probe** 10996-L24650			**Exon 3**
380	**PKD2 probe** 11001-L24655			**Exon 8**
388 «	**PKD2 probe** 14740-L24646			**Exon 1**
396	Reference probe 00713-L24645	19q13		
405 «	**PKD1 probe** 14087-L24675		**Exon 37**	
416 «	**PKD1 probe** 10990-L24673		**Exon 33**	
425	**PKD2 probe** 11008-L24662			**Exon 15**
433	Reference probe 00680-L24640	7q34		
444 «	**PKD1 probe** 14103-L24663		**Upstream**	
452 «	**PKD1 probe** 14094-L24670		**Exon 22**	
463	**PKD2 probe** 14741-L24649			**Exon 2**
471	Reference probe 07607-L24644	15q26		

« 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.

Note: The exon numbering used in this P351-C1 P352-D1 PKD1-PKD2 product description is the exon numbering from the RefSeq transcript NM_001009944.2, for the *PKD1* gene and RefSeq transcript NM_000297.3 for *PKD2*. The exon numbering and NM sequence used is from 02/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. P351 and P352 probes arranged according to chromosomal location

Table 2a. *PKD1*

Length (nt) P351/P352	SALSA MLPA probe	PKD1 exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
			***PKD1* gene**		
			NM_001009944.2		
		start codon	*210-212 (exon 1)*		
444	14103-L24663	Upstream	2.0 kb before exon 1	TCTTCCCTGAAC-CAAACAAGATCT	0.5 kb
257	14102-L24664	Upstream	1.5 kb before exon 1	CATTGAGACTTG-GTTAATCTGTTT	18.2 kb
141	10938-L24677	Exon 3	10 nt before exon 3	CCGAATCCCCCG-TCTCTTCCAGGG	1.1 kb
297	10940-L24678	Exon 5	1143-1144	GAGACGGCTCCG-CCGAGGTGGATG	0.5 kb
185	19917-L11651	Exon 6	1519-1520	GGAGCAGTGTCA-GGCCTGGGCCGG	0.7 kb
153	14100-L24666	Exon 7	1807-1808	CTGCGAGCTGCA-GCCCGGAGGTGT	0.7 kb
394	10941-L24679	Exon 9	64 nt before exon 9	GAAGTTCGGGTA-GGGGGAGTCTGG	0.9 kb
266	10942-L27362	Exon 10	83 nt after exon 10	GGGTCTGTGCAC-CAGACACACCCA	1.0 kb
172	10943-L24681	Exon 11	2916-2917	TGCCGTGGCTCA-GTGAGGGGGAGC	1.0 kb
307	10944-L24682	Exon 12	11 nt before exon 12	GGCTGACACCAT-TCCCCCCGCAGA	0.4 kb
361	19919-L24683	Exon 13	3242-3243	AACTACAACGTA-ACCGTGGAGCGG	0.4 kb
196	10946-L27456	Exon 14	17 nt before exon 14	TCACTCACTGCG-TCCCACCGCCCC	1.0 kb
248	10983-L24667	Exon 15	3851-3852	GAGCTCCGCGGA-CTCAGCGTGGAC	1.3 kb
343	14097-L24685	Exon 15	5177-5176 reverse	GTGCCATCCCTA-ACCACGGCCTGC	1.5 kb
251	14096-L24686	Exon 15	6712-6713	ACAGCGCAACTA-CTTGGAGGCCCA	0.9 kb
239	10947-L24687	Exon 16	95 nt after exon 16	GTGGAGGCCGCA-CGCTCTCCCCTC	1.2 kb
290	10949-L24688	Exon 18	7553-7554	ACCTTCACGCTC-ACGGTGCTGGGC	0.3 kb
291	10984-L24668	Exon 19	7809-7810	GCAGCCTCTCCA-GCTACGGAGCCG	0.4 kb
154	10950-L24689	Exon 20	24 nt after exon 20	TGGGAGGGGACG-TCACATCTGCTG	0.3 kb
270	10986-L24669	Exon 21	36 nt before exon 21	CGGCCTCCTGCG-CTGCTGACAGCT	0.9 kb
452	14094-L24670	Exon 22	8296-8297	GGCCATGATGCT-CATCCTGCAGGC	0.7 kb
226	10952-L24690	Exon 23	8416-8417	GGCACCACAGCC-CTCAGAGCTGGG	1.3 kb
414	10953-L24691	Exon 25	9276-9277	GCGAGGAGGACA-TGGTGTGGCGGA	0.5 kb
315	11009-L24671	Exon 26	12 nt after exon 26	GTGAGGGGCGCA-GCGGGGTGGCAG	1.4 kb
274	10954-L24692	Exon 27	86 nt before exon 27	CTTGAGTGCGCA-CAGGCCAAAGCT	0.6 kb
322	10955-L24693	Exon 29	9935-9936	GACGCAGCCCTT-TTGCGCTTCCGG	0.6 kb
258	10956-L24694	Exon 30	196 nt after exon 30	GAAGCCAGAATG-GTGAAAGAACGA	1.6 kb
191	11011-L27419	Exon 31	7 nt after exon 31	CTGAGGTGAGGA-CTCTACTGGGGG	0.5 kb
416	10990-L24673	Exon 33	10604-10605	GCCAAATCCTTC-TCAGCATCAGGT	3.1 kb
450	19918-L24695	Exon 35	36 nt before exon 35	CTGCCTCCTGGA-GGCCGGGATGAA	0.3 kb
159	10992-L24674	Exon 36	10848-10849	AGGGTCTGCGGA-AGCGCCTGCTGC	0.4 kb
405	14087-L24675	Exon 37	11176-11177	TGCACTCTTCCT-GGCCAAGGAAGA	0.6 kb
178	10958-L24696	Exon 38	11324-11325	CAAAGCGCCATC-AAGCAGGAGCTG	0.4 kb
379	10959-L24697	Exon 39	11388-11389	TCTGGCCATGGA-TGGCCCACGTGC	0.5 kb
148	10960-L16105	Exon 40	11549-11550	AGCACCAGCGAT-TACGACGTTGGC	0.3 kb
160	10961-L24698	Exon 41	11684-11685	GAGCTGGGCCTG-AGCCTGGAGGAG	0.3 kb
202	10962-L24699	Exon 42	11766-11767	CTGTGTTCCTGG-AGCTCACGCGCT	0.4 kb
184	10963-L24700	Exon 43	11949-11950	TCGCCGTGCACT-TCGCCGTGGCCG	0.4 kb
335	10964-L27363	Exon 44	12250-12251	CCAGTGGTCCGT-CTTTGGCAAGAC	1.3 kb
232	14095-L24702	Exon 46	13364-13365	ACGGTTTCTAGC-CTCTGAGATGCT	1.2 kb
		stop codon	*13119-13121 (exon 46)*		
			***TSC2* gene**		
			NM_000548.3		
		stop codon	*5528-5530 (exon 42)*		
370	11935-L24630	exon 41	5346-5347	GCTCCGCCACAT-CAAGCGGCTCCG	1.5 kb
442	01843-L24629	exon 38 (37)	5041-5042	GGCAACGACTTT-GTGTCCATTGTC	1.5 kb
424	01842-L24628	TSC2 exon 36 (35)	4724-4725	AGATCCCATCAT-ACGACACCCACA	

		start codon	*107-109 (exon 2)*		

Note 1: The exon numbering used in this P351-C1 P352-D1 PKD1-PKD2 product description is the exon numbering from the RefSeq transcript NM_001009944.2, for the *PKD1* gene. The exon numbering and NM sequence used is from 02/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Note 2: The complete *PKD1* gene is difficult to denature but the region between PKD1 exon 31 and TSC2 exon 41 is even more difficult to denature due to an extremely high % GC. This may cause false positive results in samples containing salt (for more information: www.mlpa.com -> MLPA procedure -> Sample preparation).

Note 3: No probes are present for exons 1, 2, 4, 8, 17, 24, 28, 32, 34 and 45. This is due to the existence of several *PKD1* pseudogenes that are almost identical to the actual gene. These are also present on chromosome 16. Probes detecting exon 3-31 of *PKD1* depend for their specificity on a single nucleotide difference between the *PKD1* gene and the pseudogenes. For these probes, an apparent duplication can be the result of a clinically non-significant one nucleotide sequence change in one of these pseudogenes.

Note 4: The *TSC2* exon numbering has changed. From description version C1/D1-01 onwards, we have adopted the NCBI exon numbering that is present in the LRG for this gene. The exon numbering used in previous versions of this product description can be found between brackets in Table 2a. The exon numbering is from LRG_487 (NM_000548.3) and is from 02/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2b. *PKD2*

Length (nt)	SALSA MLPA probe	PKD2 exon	Ligation site NM_000297.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*88-90 (exon 1)*		
388 «	14740-L24646	Exon 1	102-103	GTGAACTCCAGT-CGCGTGCAGCCT	0.2 kb
297 «	10994-L24647	Exon 1	254-255	GGAGATCGAGAT-GCAGCGCATCCG	11.6 kb
142 «	10995-L24648	Exon 2	726-727	AGCACTAACCGA-GAGAAATACCTT	0.1 kb
463	14741-L24649	Exon 2	2 nt after exon 2	TGTGCATCTGTA-AGTAGAATATTT	16.2 kb
371	10996-L24650	Exon 3	425 nt before exon 3	TATTGCAGGGAG-AGTAGGCTGGTG	2.5 kb
342	10997-L24651	Exon 4	967-968	GGCTGTACTGGA-AGATGCAGCCCA	5.0 kb
220	10998-L24652	Exon 5	1276-1277	TGGATTTGTCAA-GAACAAGAGAGG	3.4 kb
263	14738-L20398	Exon 6	1493-1494	TGTCACAACTTT-TGATTTCTTCCT	0.1 kb
233	14739-L24653	Exon 6	1584-1585	CGCATTCACAAA-CTACACTATTTC	5.3 kb
202	11000-L24654	Exon 7	1708-1709	AGTTTCTGGAAG-ATCAAAATACTT	4.1 kb
380	11001-L24655	Exon 8	1849-1850	GCCAGCTCTCGA-CAACCATGTCTC	1.9 kb
325	11002-L24656	Exon 9	2048-2049	GGAAGCTAATCG-AGTTTTGGGACC	3.9 kb
352	11003-L24657	Exon 10	2164-2165	CTGACTTGGCAC-AGCAGAAAGCTG	3.5 kb
166	11004-L24658	Exon 11	2291-2292	AGGAGGAGGCAA-GTTAAACTTTGA	0.4 kb
148	11005-L24659	Exon 12	2391-2392	GATGGAGACCAA-GAACTGACCGAA	9.0 kb
	No probe	Exon 13			
178	11007-L24661	Exon 14	2663-2664	CATAGTGTCCAA-GATTGACGCCGT	0.7 kb
425	11008-L24662	Exon 15	2860-2861	CAGCTTCCCAGA-TCAGTCATGGTT	
		stop codon	*2992-2994 (exon 15)*		

« 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.

Note: The exon numbering used in this P351-C1 P352-D1 PKD1-PKD2 product description is the exon numbering from the RefSeq transcript NM_000297.3 for *PKD2*. The exon numbering and NM sequence used is from 02/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

P046 TSC2	Contains probes for the *TSC2* gene
P337 TSC2	Contains probes for the *TSC2* gene, used as confirmation of P046 results
P341-342 PKDH1	Contains probes for the *PKHD1* gene, responsible for the recessive form of PKD

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- Simms RJ et al. (2015). Genetic testing in the assessment of living related kidney donors at risk of autosomal dominant polycystic kidney disease. *Transplantation*. 99:1023-1029.
- Yu G et al. (2015). Analysis of gene mutations in PKD1/PKD2 by multiplex ligation-dependent probe amplification: some new findings. *Ren Fail*. 37:366-371.

P351 Product history	
Version	*Modification*
C1	One target probe has been removed, three reference probes have been replaced.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	Three extra PKD1 probes have been included.
A1	First release.

P352 Product history	
Version	*Modification*
D1	One target probe has been removed and two reference probes replaced.
C1	Four new PKD2 probes have been added, one PKD2 probe removed. QDX2 has replaced QDX

	fragments.
B1	Four extra PKD1 probes have been included.
A1	First release.

Implemented changes in the product description
Version C1/D1-01 – 08 March 2018 (01P) - Product description restructured and adapted to a new template. - Various minor textual and layout changes. - Exon numbering *TSC2* adjusted *Version 08 - 30 March 2015 (54)* - Product description adapted to a new product version (version number changed, lot number added, changes in Table 2 and Table 3, new picture included). *Version 07 (48)* - Warning added in Table 1, 427 nt probe 10297-L10809. *Version 06 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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