Product Description SALSA® MLPA® Probemix P341-B4/P342-C1 PKHD1

To be used with the MLPA General Protocol.

P341 version B4. As compared to version B3, two reference probes have been replaced and one reference probe has been added. For complete product history see page 9.
P342 version C1. As compared to version B3, one probe for *PKHD1* has been removed and two reference probes have been replaced. For complete product history see page 9.

Catalogue numbers:
- **P341-025R:** SALSA MLPA Probemix P341 PKHD1 mix 1, 25 reactions.
- **P341-050R:** SALSA MLPA Probemix P341 PKHD1 mix 1, 50 reactions.
- **P341-100R:** SALSA MLPA Probemix P341 PKHD1 mix 1, 100 reactions.

- **P342-025R:** SALSA MLPA Probemix P342 PKHD1 mix 2, 25 reactions.
- **P342-050R:** SALSA MLPA Probemix P342 PKHD1 mix 2, 50 reactions.
- **P342-100R:** SALSA MLPA Probemix P342 PKHD1 mix 2, 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.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 P341/P342 PKHD1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PKHD1* gene, which is associated with autosomal recessive polycystic kidney disease (ARPKD).

ARPKD is a hereditary and severe form of polycystic kidney disease, affecting the kidneys and the hepatic biliary tract. The clinical spectrum is widely variable, with most cases presenting during infancy. The fetal phenotypic features classically include enlarged and echogenic kidneys, as well as oligohydramnios secondary to a poor urine output. Up to 50% of the affected neonates die shortly after birth, as a result of severe pulmonary hypoplasia and secondary respiratory insufficiency. In the subset that survives the perinatal period, morbidity and mortality are mainly related to severe systemic hypertension, renal insufficiency, and portal hypertension due to portal-tract fibrosis. Defects in the *PKHD1* gene are the cause of all typical forms of ARPKD.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1326/.

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/

Exon numbering: The *PKHD1* exon numbering used in this P341-B4/P342-C1 PKHD1 product description is the exon numbering from the RefSeq transcript NM_138694.4, which is identical to the NG_008753.1

sequence. The exon numbering and NM_ sequence used have been retrieved on 04/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P341-B4 PKHD1 mix 1 contains 44 MLPA probes with amplification products between 136 and 472 nucleotides (nt). This includes 35 probes for the *PKHD1* gene and nine reference probes that detect autosomal chromosomal locations. The SALSA MLPA Probemix P342-C1 PKHD1 mix 2 contains 44 MLPA probes with amplification products between 130 and 463 nucleotides (nt). This also includes 35 probes for the *PKHD1* gene and nine reference probes that detect autosomal chromosomal locations. Both probemixes together include 70 probes targeting 65 out of the 67 exons of the *PKHD1* gene, one probe for each exon of the gene with the exception of exon 17 and 44, and two probes are present for exons 27, 35, 36, 37, and 53. Complete probe sequences and the identity of the genes detected by the reference probes are 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 nt and 96 nt 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 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 unrelated individuals who are from families without a history of kidney diseases. 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 Institute (https://catalog.coriell.org) and Leibniz Institute 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.

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 each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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. 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:
- In most populations, the major cause of genetic defects in the *PKHD1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P341/P342 PKHD1.
- 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.

***PKHD1* mutation database:** https://databases.lovd.nl/shared/genes/PKHD1. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *PKHD1* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P341-B4 PKHD1 mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*PKHD1*
64-105	Control fragments – see table in probemix content section for more information		
136	Reference probe 08030-L07811	11q24	
142	**PKHD1 probe** 10679-L11261		**Exon 34**
148	**PKHD1 probe** 10663-L11245		**Exon 1**
154	**PKHD1 probe** 10694-L11276		**Exon 60**
160	**PKHD1 probe** 20958-L11255		**Exon 21**
166	**PKHD1 probe** 10671-L11253		**Exon 16**
172	**PKHD1 probe** 12185-L13107		**Exon 35**
178	**PKHD1 probe** 10676-L11258		**Exon 28**
185	**PKHD1 probe** 10666-L11248		**Exon 7**
190	Reference probe 09953-L10412	17p13	
197	**PKHD1 probe** 10688-L11270		**Exon 49**
202	**PKHD1 probe** 10697-L12697		**Exon 67**
209	**PKHD1 probe** 10665-L11247		**Exon 4**
218	**PKHD1 probe** 10695-L24064		**Exon 61**
226	**PKHD1 probe** 10672-L11254		**Exon 20**
232	**PKHD1 probe** 20959-L29093		**Exon 53**
238	**PKHD1 probe** 10668-L11250		**Exon 11**
244	**PKHD1 probe** 10683-L11265		**Exon 40**
250 *	Reference probe 06387-L21633	8p23	
257	**PKHD1 probe** 10692-L11274		**Exon 57**
265	**PKHD1 probe** 10674-L11256		**Exon 23**
274	**PKHD1 probe** 10696-L11278		**Exon 64**
283	**PKHD1 probe** 10680-L11262		**Exon 35**
292 *	Reference probe 08722-L28962	9q21	
301	**PKHD1 probe** 10669-L11251		**Exon 14**
310	**PKHD1 probe** 10689-L11271		**Exon 50**
319	**PKHD1 probe** 10677-L11259		**Exon 29**
328	**PKHD1 probe** 10682-L11264		**Exon 37**
337	Reference probe 07722-L07432	7p13	
346	**PKHD1 probe** 10664-L11246		**Exon 3**
355	**PKHD1 probe** 10684-L11266		**Exon 42**
364	**PKHD1 probe** 10675-L11257		**Exon 25**
373	**PKHD1 probe** 10693-L11275		**Exon 58**
382	Reference probe 13055-L14238	15q14	
391 ±	**PKHD1 probe** 10691-L11273		**Exon 55**
400	**PKHD1 probe** 10670-L11252		**Exon 15**
409	**PKHD1 probe** 10681-L11263		**Exon 36**
418	**PKHD1 probe** 10687-L11269		**Exon 46**
427	Reference probe 10035-L11450	2q37	
436 +	**PKHD1 probe** 10678-L11260		**Exon 33**
445	**PKHD1 probe** 10667-L11249		**Exon 8**
454	**PKHD1 probe** 10685-L11267		**Exon 43**
463 *	Reference probe 18379-L23434	12p11	
472	Reference probe 12761-L13877	4q12	

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

* New in version B4.
± SNP rs150925674 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
+ SNP rs770522674 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 1b. SALSA MLPA Probemix P342-C1 PKHD1 mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*PKHD1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 05169-L04550	8q24	
136	**PKHD1 probe** 10719-L11301		**Exon 41**
142	**PKHD1 probe** 10699-L11281		**Exon 5**
148	Reference probe 04730-L04147	7q21	
155	**PKHD1 probe** 10714-L11296		**Exon 32**
160	**PKHD1 probe** 10686-L11268		**Exon 45**
166	**PKHD1 probe** 10729-L24067		**Exon 62**
172	**PKHD1 probe** 10701-L11283		**Exon 9**
178	**PKHD1 probe** 10722-L11304		**Exon 48**
184	**PKHD1 probe** 10710-L11292		**Exon 26**
190 *	Reference probe 21401-L31194	3q22	
196	**PKHD1 probe** 10723-L11305		**Exon 51**
204	**PKHD1 probe** 10712-L29205		**Exon 30**
211	**PKHD1 probe** 10730-L29204		**Exon 63**
217	**PKHD1 probe** 10698-L29206		**Exon 2**
226	Reference probe 10433-L29207	9q34	
230	**PKHD1 probe** 10721-L29208		**Exon 47**
238	**PKHD1 probe** 10724-L11306		**Exon 52**
244	**PKHD1 probe** 10716-L11298		**Exon 37**
256	**PKHD1 probe** 10702-L11284		**Exon 10**
265	**PKHD1 probe** 10728-L11310		**Exon 59**
274	**PKHD1 probe** 10708-L11290		**Exon 22**
283	**PKHD1 probe** 10732-L11314		**Exon 66**
292	**PKHD1 probe** 11900-L12706		**Exon 19**
301	**PKHD1 probe** 10726-L11308		**Exon 54**
310	**PKHD1 probe** 10704-L11286		**Exon 13**
317	**PKHD1 probe** 11898-L24065		**Exon 12**
325	**PKHD1 probe** 10711-L24066		**Exon 27**
337	Reference probe 09937-L12248	8q13	
346	**PKHD1 probe** 10713-L11295		**Exon 31**
355	**PKHD1 probe** 10727-L11309		**Exon 56**
364 *	Reference probe 06348-L05863	1p21	
373	**PKHD1 probe** 10718-L11300		**Exon 39**
382	**PKHD1 probe** 11901-L12707		**Exon 27**
391	**PKHD1 probe** 10725-L11307		**Exon 53**
400	**PKHD1 probe** 20685-L22083		**Exon 36**
409	Reference probe 09999-L10331	20q13	
416	**PKHD1 probe** 20960-L29094		**Exon 6**
422	**PKHD1 probe** 12187-L29298		**Exon 18**
427	Reference probe 15893-L24326	2p16	
437	**PKHD1 probe** 10731-L11313		**Exon 65**
445	**PKHD1 probe** 10709-L11291		**Exon 24**
454	**PKHD1 probe** 10717-L11299		**Exon 38**
463	Reference probe 09908-L10321	16p13	

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

* New in version C1.

Table 2. PKHD1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*PKHD1* exon[a]	Ligation site NM_138694.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*265-267 (Exon 2)*		
148	10663-L11245	Exon 1	81-82	CTCTAACCAGAT-AACATGTCCACG	2.6 kb
217	10698-L29206	Exon 2	296-297	TCTGATGAGTAT-TGAAGTACTACT	1.7 kb
346	10664-L11246	Exon 3	348-349	ATTGAACCTGAA-GAAGGTAGCCTT	0.7 kb
209	10665-L11247	Exon 4	436-437	ATGGCTCTCAAT-TGGAGATACACC	2.5 kb
142	10699-L11281	Exon 5	580-581	AGGGTCTGTACT-TCCTGGAAGCAT	3.7 kb
416	20960-L29094	Exon 6	700-701	TTTATCCACCAA-GTGGTGTTCCAG	2.8 kb
185	10666-L11248	Exon 7	759-760	GGAAGATTGGAA-ACTTTTGATTTT	1.3 kb
445	10667-L11249	Exon 8	821-822	TCAAGGAGACAA-ATGGGTTACTCC	1.1 kb
172	10701-L11283	Exon 9	8 nt before exon 9	TCAGTTTGTCTT-TTCCTTAGTTAT	0.7 kb
256	10702-L11284	Exon 10	959-960	CTTCTCAGTATT-TAACAAAGGAAA	0.9 kb
238	10668-L11250	Exon 11	1001-1002	GGCATGGCTGAT-CAGTGCTAAACA	3.4 kb
317	11898-L24065	Exon 12	1065-1066	GTGTTTCCAGAA-ACTGGGAGCCTT	1.0 kb
310	10704-L11286	Exon 13	1184-1185	TCCCAGGAAGAT-TGAGTGCACCAC	2.5 kb
301	10669-L11251	Exon 14	1349-1350	CAGTTCTCCATT-TGGGTTTTGGTC	2.6 kb
400	10670-L11252	Exon 15	1479-1480	TTCAGTTGGTCA-GAGGAACCAAGG	1.5 kb
166	10671-L11253	Exon 16	1699-1700	ACACCTGGCTGA-ATCCTGATGTGG	1.7 kb
	No probe	Exon 17			
422	12187-L29298	Exon 18	1906-1907	CAGTAAAATGCA-AACTGGAACCCC	1.2 kb
292	11900-L12706	Exon 19	61 nt after exon 19	TGCTCTCTGGAT-TCAAGACTGAAA	1.4 kb
226	10672-L11254	Exon 20	2128-2129	AAGGCCACATGA-ACAAGATCCTGA	0.9 kb
160	20958-L11255	Exon 21	2263-2264	TCTGGGAGACTT-GTGTGCGTTGCT	2.9 kb
274	10708-L11290	Exon 22	2418-2419	TCTCAAGCTGAT-TCTGGAACGGCT	1.7 kb
265	10674-L11256	Exon 23	2558-2559	TGTGCCCACTGA-AGGAACAGAAGA	2.4 kb
445	10709-L11291	Exon 24	2688-2689	CCTGTACAAATT-TCTGCTCATCAC	1.2 kb
364	10675-L11257	Exon 25	2926-2927	ATGGTGGAGTTT-TTCTTGGACCCA	1.3 kb
184	10710-L11292	Exon 26	3030-3031	GGTTCCTGCTCT-TTCCAGTACCTC	0.6 kb
382	11901-L12707	Exon 27	3167-3168	TACAGTGAACAA-AACGAGTTGCAA	0.1 kb
325	10711-L24066	Exon 27	3254-3255	TCGGATCTTGAT-GTTGGTGAGACC	7.4 kb
178	10676-L11258	Exon 28	3459-3460	GCTACAAGCAAT-TCAAGCAGAATT	2.5 kb
319	10677-L11259	Exon 29	3522-3523	GTGAATGTGACT-GTGATCAGAGGG	4.8 kb
204	10712-L29205	Exon 30	3679-3680	ACTATACGGATT-TGGATGTGGAAG	0.4 kb
346	10713-L11295	Exon 31	3857-3858	CCTCACAGAAGT-TTTCAGCATCGA	2.4 kb
155	10714-L11296	Exon 32	4562-4563	TACTTGTGTGAT-TTTGAGTTTGGG	2.7 kb
436 +	10678-L11260	Exon 33	5604-5605	CTGGCTAATGCT-ACAGTGTCTGCC	5.4 kb
142	10679-L11261	Exon 34	5790-5791	ATTTGCGAGGAA-AGTTCCCAATGC	7.0 kb
172	12185-L13107	Exon 35	22 nt before exon 35	ATGACACCCCAT-TTAACCTCCCCT	0.1 kb
283	10680-L11262	Exon 35	5966-5967	CAATCAGCCAAT-TACCGTCAAGAT	50.4 kb
409	10681-L11263	Exon 36	6068-6069	TCACAGCTGGTT-TCCTGAAAGGCT	0.1 kb
400	20685-L22083	Exon 36	6126-6127	GGCCAATTGCTT-CTGCTGGACACT	25.7 kb
244	10716-L11298	Exon 37	6234-6235	GCCATCCTTGTT-TCTGATGGTGGA	0.1 kb
328	10682-L11264	Exon 37	6302-6303	TCAGATCACACT-CTACGGGAGTTC	21.6 kb
454	10717-L11299	Exon 38	6423-6424	TGTCTTAGAGCA-ACTGCCCATGCC	0.7 kb
373	10718-L11300	Exon 39	6680-6681	CAGCAGGAGTAT-TACCATACAAGG	2.5 kb
244	10683-L11265	Exon 40	6882-6883	GGAGTGCAGTTT-CAAGTCTTGGGG	3.0 kb
136	10719-L11301	Exon 41	6969-6970	CAGGGCTGCACA-GTGAGGAACTCC	2.3 kb
355	10684-L11266	Exon 42	7095-7096	GAGATGAGATAT-ATCTCCTGGGAG	0.4 kb
454	10685-L11267	Exon 43	7233-7234	TCTGGCATCTAT-ATCTGCAGTCCC	17.7 kb
	No probe	Exon 44			
160	10686-L11268	Exon 45	7448-7449	GTTCCAGAGCTT-CACAGTTTGGGA	2.8 kb
418	10687-L11269	Exon 46	7577-7578	AAATACTTCAGT-TACTGACAGCTT	12.5 kb
230	10721-L29208	Exon 47	7643-7644	ATCTGGGATTAA-AACTCCTAAAAG	2.5 kb
178	10722-L11304	Exon 48	7789-7790	TGAAGTTTACAA-ACTCTTCAAACT	12.1 kb
197	10688-L11270	Exon 49	8124-8125	CTCTTGGACCAA-GAGACCTACTCA	8.1 kb
310	10689-L11271	Exon 50	8296-8297	TGTCTTTTCCAT-TTCTTCCATCAC	11.4 kb
196	10723-L11305	Exon 51	8399-8400	AGTTCAAGTCAT-TCTCCGGGTGAA	5.5 kb
238	10724-L11306	Exon 52	8526-8527	GGATACAACAAT-ACCATTCCAGGC	39.6 kb

Length (nt)	SALSA MLPA probe	*PKHD1* exon[a]	Ligation site NM_138694.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
232	20959-L29093	Exon 53	8594-8595	GGATACAGATCT-TCCATTCTTCAA	0.1 kb
391	10725-L11307	Exon 53	8675-8676	TGTGGCATGCAT-GGTCATTGCAGG	15.4 kb
301	10726-L11308	Exon 54	8774-8775	AGAGGGAGTCTT-TTGTGACCGTAT	3.1 kb
391 ±	10691-L11273	Exon 55	8845-8846	TTCATCTTTACA-GTGCTTATCCTA	17.9 kb
355	10727-L11309	Exon 56	8985-8986	GAGCCTCATGAA-GCAGAGGTCCTC	1.6 kb
257	10692-L11274	Exon 57	9134-9135	GACCCGAAATAT-ACAAATTCAGCC	4.7 kb
373	10693-L11275	Exon 58	9273-9274	TTGTACTCATCT-GTTGAATTCAGT	1.8 kb
265	10728-L11310	Exon 59	10161-10162	ATTCTACCAAAT-GCAGAGAACAGT	2.4 kb
154	10694-L11276	Exon 60	10375-10376	CAGTTTCTGTAT-TTCCTAAAACAG	84.9 kb
218	10695-L24064	Exon 61	10871-10872	TGCCAACTATTT-CAACATCATGGA	10.4 kb
166	10729-L24067	Exon 62	11516-11517	CCAGCCTTCAGA-TGGAGAAGTGGG	1.1 kb
211	10730-L29204	Exon 63	11637-11638	GCTTCCCTGGAA-GGAGCATCAGAC	9.1 kb
274	10696-L11278	Exon 64	11685-11686	GCAGAAACTCAA-GATGGTTATGTT	6.3 kb
437	10731-L11313	Exon 65	11825-11826	TGTGACTAGGAA-GGAGAAGTCGAC	5.7 kb
283	10732-L11314	Exon 66	7 nt after exon 66	AGAAGGTAAGCT-TGGAGGGTGGAG	7.7 kb
202	10697-L12697	Exon 67	12307-12308	TGGCAGGCCAAA-ATCAGCTGCTGC	
		stop codon	*12487-12489 (Exon 67)*		

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

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

± SNP rs150925674 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
+ SNP rs770522674 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P341/P342 PKHD1

- Koczok K et al. (2018). Interfering effect of maternal cell contamination on invasive prenatal molecular genetic testing. Prenatal diagnosis, 38(9), 713-719.
- Lazaros L et al. (2019). Identification of a Novel Intragenic Deletion of the PHKD1 Gene in a Patient with Autosomal Recessive Polycystic Kidney Disease. International Journal of Pediatrics, 7(10), 10291-10297.
- Melchionda S et al. (2016). Expanding the mutation spectrum in 130 probands with ARPKD: identification of 62 novel PKHD1 mutations by sanger sequencing and MLPA analysis. J Hum Genet. Epub 2016 May 26. doi: 10.1038/jhg.2016.58.
- Miyazaki J et al. (2015). Intragenic duplication in the PHKD1 gene in autosomal recessive polycystic kidney disease. *BMC Med Genet.* 16(98): 2-6.
- Obeidova L et al. (2015). Molecular genetic analysis of PKHD1 by next-generation sequencing in Czech families with autosomal recessive polycystic kidney disease. *BMC Med Genet.* 16(116):1-12.
- Szabó T et al. (2018). Comprehensive genetic testing in children with a clinical diagnosis of ARPKD identifies phenocopies. Pediatric Nephrology, 33(10), 1713-1721.
- Zvereff V et al. (2010). Identification of PKHD1 multiexon deletions using multiplex ligation-dependent probe amplification and quantitative polymerase chain reaction. *Genet Test Mol Biomarkers.* 14:505-10.

P341 Product history	
Version	*Modification*
B4	Two reference probes have been replaced and one reference probe has been added.
B3	The lengths of several probes have been adjusted.
B2	Two reference probes have been removed and control fragments have been adjusted (QDX2).
B1	Extra exon 35 probe, X and Y fragments added, and several reference probes have been replaced.
A1	First release.

P342 Product history	
Version	*Modification*
C1	One probe for *PKHD1* has been removed and two reference probes have been replaced.
B3	The lengths of several probes have been adjusted.
B2	One reference probe has been replaced, one additional reference probe has been included, and the control fragments have been adjusted (QDX2).
B1	Exon 18 probe added, exon 17 probe removed, X and Y fragments added, and one reference probe removed.
A1	First release.

Implemented changes in the product description

Version B4/C1-01 — 12 June 2020 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *PKHD1* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Warning added to Tables 1 and 2 about specific SNPs that could influence the probe signal.

Version 08 (55)- 28 June 2016
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes.
- New references added on page 2.
- PKHD1 exon numbering adjusted.

Version 07 (49)
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.

Version 06 (49)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 05 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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