Product Description
SALSA® MLPA® Probemix P474-A1 CD274-PDCD1LG2-JAK2

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 10.

Catalogue numbers:
- **P474-025R:** SALSA MLPA Probemix P474 CD274-PDCD1LG2-JAK2, 25 reactions.
- **P474-050R:** SALSA MLPA Probemix P474 CD274-PDCD1LG2-JAK2, 50 reactions.
- **P474-100R:** SALSA MLPA Probemix P474 CD274-PDCD1LG2-JAK2, 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.

General information
The SALSA MLPA Probemix P474 CD274-PDCD1LG2-JAK2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *CD274* (also known as *PDCD1LG1* or *PDL1*), *PDCD1LG2* (also known as *PDL2*) and *JAK2* genes located on 9p24.1.

Amplification of the *CD274* gene, often co-amplified with *PDCD1LG2* and *JAK2*, has been described in various types of cancer. In nearly all cases of classical Hodgkin lymphoma the copy number of *CD274* and *PDCD1LG2* is increased, either by amplification, copy gain or polysomy, but only amplification of 9p24.1 correlates with shorter progression-free survival (Roemer et al. 2016). Likewise, *JAK2* copy number is increased in 64% of classical Hodgkin lymphoma cases (Van Roosbroeck et al. 2016).

In addition, in various subtypes of lung cancer, amplification of 9p24.1 is common; in 40-43% of pulmonary squamous-cell and adenocarcinomas, in 5-16% of non-small cell lung cancer (NSCLC) and in 2-5% of small cell lung cancer *CD274*, *PDCD1LG2* and/or *JAK2* copy number is increased (Clavé et al. 2018; Goldmann et al. 2016; Ikeda et al. 2015; Inoue et al. 2016; George et al. 2016; Iwakawa et al. 2013). In non-squamous NSCLC CD274 copy number may predict different response to immune checkpoint inhibitor treatment (Murugesan et al. 2022).

In certain types of soft-tissue sarcoma *CD274* copy number gains are also common, for example in 34-60% of undifferentiated pleomorphic sarcomas and in 35% of myxofibrosarcomas (Boxberg et al. 2017; Budczies et al. 2017). But also in 29% of triple negative breast carcinomas, in 19% of oral squamous cell carcinomas, in 5% of glioblastomas, in 3% of anaplastic thyroid carcinomas, and in 1-3% of colon carcinomas an increased copy number of *CD274*, *PDCD1LG2* and/or *JAK2* is found (Barrett et al. 2015; Straub et al. 2016; Pozdeyev et al. 2018; Lee et al. 2018).

Interestingly, in a large study on 22 major cancer types it became apparent that deletions of *CD274* are more frequent (31%) than amplifications (12%) and both types of copy number alterations are associated with dismal prognosis (Budczies et al. 2016).

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 exon numbering used in this P474-A1 CD274-PDCD1LG2-JAK2 product description is the exon numbering from NM_014143.4 for *CD274*, NM_025239.4 for *PDCD1LG2* and LRG_612 for *JAK2*. 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 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 P474-A1 CD274-PDCD1LG2-JAK2 contains 51 MLPA probes with amplification products between 124 and 500 nucleotides (nt). This includes seven probes for the *CD274* gene, 10 probes for the *PDCD1LG2* gene and 12 probes for the *JAK2* gene. In addition, 17 reference probes are included that target relatively copy number stable regions in various cancer types. Probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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, which includes DNA derived from paraffin-embedded tissues, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

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 healthy individuals without a history of cancer. 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 NA10989, NA14946, NA02819, NA03226, NA05067 and NA13480 from the Coriell Institute have been tested with this P474-A1 probemix at MRC Holland and can be used as a positive control samples to detect the copy number alterations mentioned 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 (hg18)*	Altered target gene(s) in P474-A1	Expected copy number alteration
NA10989	Coriell Institute	9p24.1-24.3	*SMARCA2*, *VLDLR*, *JAK2*, *CD274* and *PDCD1LG2*	Heterozygous deletion
NA14946	Coriell Institute	9p24.1-24.3	*SMARCA2*, *VLDLR*, *JAK2*, *CD274* and *PDCD1LG2*	Heterozygous deletion
NA02819	Coriell Institute	9p13.3-24.3	*SMARCA2*, *VLDLR*, *JAK2*, *CD274*, *PDCD1LG2*, *KLHL9* and *DNAI1*	Heterozygous duplication
NA03226	Coriell Institute	9p13.3-24.3	*SMARCA2*, *VLDLR*, *JAK2*, *CD274*, *PDCD1LG2*, *KLHL9* and *DNAI1*	Heterozygous duplication
NA05067	Coriell Institute	9p13.3-24.3	*SMARCA2*, *VLDLR*, *JAK2*, *CD274*, *PDCD1LG2*, *KLHL9* and *DNAI1*	Heterozygous duplication
NA13480	Coriell Institute	9p24.1	*JAK2* exon 23 and 24	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 P474-A1 CD274-PDCD1LG2-JAK2 probemix.

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 standard deviation of each individual probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the final ratio (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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- 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. 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.

P474 specific note:

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Limitations of the procedure

- In most populations, most genetic alterations in *CD274*, *PDCD1LG2* and *JAK2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P474 CD274-PDCD1LG2-JAK2.

- 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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in solid tumours with more chaotic karyotypes.

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.

COSMIC mutation database

http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the Catalogue Of Somatic Mutations In Cancer (COSMIC). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *CD274* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P474-A1 CD274-PDCD1LG2-JAK2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					Location (hg18) in kb
		Reference	CD274	PDCD1LG2	JAK2	Flanking	
64-105	Control fragments – see table in probemix content section for more information						
124	Reference probe 19616-L26275	4p13					04-042,278
130	Reference probe 13867-L15385	16p13					16-008,765
137	**JAK2 probe** 21199-L29574				**Exon 4**		09-005,020
142	**CD274 probe** 21203-L29578		**Exon 4**				09-005,453
148	**CD274 probe** 21546-L30645		**Exon 1**				09-005,441
154	**PDCD1LG2 probe** 21201-L29576			**Exon 4**			09-005,539
160	**JAK2 probe** 21202-L29577				**Exon 3**		09-005,012
166	Reference probe 13816-L30646	2q13					02-108,906
172	Reference probe 21193-L29851	6p12					06-052,046
178	**JAK2 probe** 21204-L29579				**Exon 6**		09-005,041
184	**PDCD1LG2 probe** 21205-L29852			**Exon 1**			09-005,501
190	**JAK2 probe** 21206-L29581				**Exon 1**		09-004,975
196	Reference probe 05703-L29853	3q21					03-123,456
202	**PDCD1LG2 probe** 21207-L29582			**Exon 2**			09-005,512
209	**JAK2 probe** 21208-L29854				**Exon 7**		09-005,045
218	Reference probe 09103-L31000	4q25					04-110,901
223	**CD274 probe** 21209-L29855		**Exon 2**				09-005,446
229 « ¬	SMARCA2 probe 06654-L29856					9p24.3	09-002,023
235	Reference probe 15918-L30940	5q31					05-131,742
244 «	**JAK2 probe** 21210-L29585				**Exon 16**		09-005,068
250	**PDCD1LG2 probe** 21211-L29586			**Exon 2**			09-005,513
257	**CD274 probe** 21212-L29857		**Exon 6**				09-005,457
265	Reference probe 08938-L09033	11p15					11-020,596
274	**PDCD1LG2 probe** 21213-L29588			**Exon 6**			09-005,553
283 «	**JAK2 probe** 21214-L29589				**Exon 22**		09-005,081
292 ¬	DNAI1 probe 08062-L07843					9p13.3	09-034,475
301	**PDCD1LG2 probe** 21215-L29590			**Exon 7**			09-005,560
310	Reference probe 06719-L06305	15q24					15-070,427
317	Reference probe 06580-L30649	2q24					02-165,907
325	**JAK2 probe** 21217-L30064				**Exon 23**		09-005,113
331	**PDCD1LG2 probe** 22022-L30938			**Exon 5**			09-005,548
340	Reference probe 05388-L24168	12p11					12-032,895
346	**CD274 probe** 21219-L29859		**Exon 3**				09-005,447
355	Reference probe 11377-L12102	17q21					17-045,601
364 ¬	KLHL9 probe 16746-L19357					9p21.3	09-021,322
373	**PDCD1LG2 probe** 21220-L29595			**Exon 3**			09-005,525
382	**JAK2 probe** 21221-L29596				**Exon 2**		09-004,976
392	**PDCD1LG2 probe** 21222-L29597			**Exon 3**			09-005,525
400	Reference probe 14839-L16547	1p34					01-045,253
409	**JAK2 probe** 21223-L29598				**Exon 24**		09-005,116
418	**CD274 probe** 21224-L29599		**Exon 7**				09-005,458
427	Reference probe 08839-L22026	2p13					02-071,767
436	**PDCD1LG2 probe** 21225-L29600			**Exon 1**			09-005,501
444	**JAK2 probe** 21226-L29601				**Exon 9**		09-005,055
454	**CD274 probe** 21227-L29602		**Exon 5**				09-005,456
463	Reference probe 21194-L29549	14q11					14-022,972
470 ¬	VLDLR probe 20332-L29860					9p24.2	09-002,644
477 ¬	PCSK5 probe 20580-L14666					9**q**21.13	09-077,939
484	**JAK2 probe** 21228-L29603				**Exon 5**		09-005,034
493	Reference probe 09682-L30803	3p25					03-008,762
500	Reference probe 21229-L29604	10p11					10-032,800

[a] See section Exon numbering on page 2 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.
¬ 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. Single probe aberration(s) must be confirmed by another method.

Table 2. P474-A1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Flanking probes telomeric of *JAK2*.					
229 « ¬	06654-L29856	*SMARCA2*	9p24.3	AAGGGCACTGGT-ATGCGACCACCT	0.6 **M**b
470 ¬	20332-L29860	*VLDLR*	9p24.2	CTATACTTCTTC-TTTTCCACAGAT	2.3 **M**b
JAK2, at 9p24.1. Indicated ligation sites are in NM_004972.4.					
190	21206-L29581	Exon 1	191-192	GTCCTCGCTGCC-GAGGGATGTGAG	0.5 kb
382	21221-L29596	Exon 2	400-401	GTGAACTGTTTC-TCTTCTGCAGAA	36.0 kb
		start codon	*468-470 (exon 3)*		
160	21202-L29577	Exon 3	482-483	GGAATGGCCTGC-CTTACGATGACA	7.9 kb
137	21199-L29574	Exon 4	780-779 reverse	GGTTGACTCATC-TATATGGAAGAC	14.6 kb
484	21228-L29603	Exon 5	888-889	CTCGAGGTGCTG-AAGCTCCTCTTC	6.3 kb
178	21204-L29579	Exon 6	1035-1036	TGATGAGAATAG-CCAAAGAAAACG	3.8 kb
209	21208-L29854	Exon 7	1115-1116	TGTATTCGAGCA-AAGATCCAAGAC	10.3 kb
444	21226-L29601	Exon 9	1547-1548	AGCTCATTAAGG-GAAGCTTTGTCT	13.4 kb
244 «	21210-L29585	Exon 16	2491-2492	TGGGAATGTATG-TGCCAAAAATAT	12.5 kb
283 «	21214-L29589	Exon 22	3487-3488	AGACAAAGAATA-CTATAAAGTAAA	32.2 kb
325	21217-L30064	Exon 23	3610-3609 reverse	CAATGTATGTGA-AAAGTTCATACA	3.3 kb
409	21223-L29598	Exon 24	3683-3684	AAACAAGGACAG-ATGATCGTGTTC	0.3 **M**b
		stop codon	*3864-3866 (exon 25)*		
CD274, at 9p24.1. Indicated ligation sites are in NM_014143.4.					
148	21546-L30645	Exon 1	1 nt after exon 1 reverse	CAACGCTCCCTA-CCTGCAGGCGGA	5.6 kb
		start codon	*70-72 (exon 2)*		
223	21209-L29855	Exon 2	116-117	CTGGCATTTGCT-GAACGGTAAGAC	1.1 kb
346	21219-L29859	Exon 3	292-293	AGGAAGACCTGA-AGGTTCAGCATA	5.8 kb
142	21203-L29578	Exon 4	653-654	CAATGTGACCAG-CACACTGAGAAT	2.6 kb
454	21227-L29602	Exon 5	835-836	GTGTAGCACTGA-CATTCATCTTCC	1.2 kb
257	21212-L29857	Exon 6	912-913	AACTCAAAGAAG-CAAAGTGGTAAG	1.2 kb
		stop codon	*940-942 (exon 7)*		
418	21224-L29599	Exon 7	1097-1096 reverse	CTCCTCTGCTTT-CGCCAGGTTCCA	42.5 kb
PDCD1LG2, at 9p24.1. Indicated ligation sites are in NM_025239.4.					
436	21225-L29600	Exon 1	5-6	TTGACTCACTCT-CATGTTACGGCA	0.3 kb
184	21205-L29852	Exon 1	267-268	GCTGTGGCAAGT-CCTCATGTGAGT	11.7 kb
202	21207-L29582	Exon 2	7 nt before exon 2	TTTCAATTGTCT-ATTTCAGATCAA	0.1 kb
		start codon	*288-290 (exon 2)*		
250	21211-L29586	Exon 2	337-336 reverse	TCTTACCTGCTA-TCTGGTGAAGCT	12.2 kb
392	21222-L29597	Exon 3	380-381	GAACTGTACATA-ATAGAGCATGGC	0.2 kb
373	21220-L29595	Exon 3	625-626	CTGGGACTACAA-GTACCTGACTCT	14.4 kb
154	21201-L29576	Exon 4	743-744	CCTCTGGCAGAA-GTATCCTGGCCA	8.3 kb
331	22022-L30938	Exon 5	1046-1047	AAGCTGTATTCT-TCAAAAGGTAAG	5.4 kb
274	21213-L29588	Exon 6	1079-1080	CCTGTCACCACA-ACAAAGAGGGAA	6.9 kb
		stop codon	*1107-1109 (exon 7)*		
301	21215-L29590	Exon 7	1194-1195	GTGGCCTGCAGA-GCTTGCCATTTG	15.8 **M**b

Flanking probes centromeric of *PDCD1LG2*.					
364 ¬	16746-L19357	*KLHL9*	9p21.3	TGTGCATCCTGA-GCTGTGTGATCT	13.2 **M**b
292 ¬	08062-L07843	*DNAI1*	9p13.3	TTCACTCGGATT-TTGACAGCCAAC	43.5 **M**b
477 ¬	20580-L14666	*PCSK5*	9**q**21.13	ATTTGAACGCTA-ATGACTGGAAAA	-

[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.

« 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.
¬ 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. Single probe aberration(s) must be confirmed by another method.

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
400	14839-L16547	*UROD*	1p34	GGCTATGAGGTG-GTTGGGCTTGAC	01-045,253
427	08839-L22026	*DYSF*	2p13	TGCCATGAAGCT-GGTGAAGCCCTT	02-071,767
166	13816-L30646	*EDAR*	2q13	CTCACATTCCTT-GGTGTTGGGGGG	02-108,906
317	06580-L30649	*SCN2A*	2q24	AACTTGGTTTGG-CAAATGTGGAAG	02-165,907
493	09682-L30803	*CAV3*	3p25	GCATCAGCCACA-TCTACTCACTCT	03-008,762
196	05703-L29853	*CASR*	3q21	GTGGCTTCCAAA-GACTCAAGGACC	03-123,456
124	19616-L26275	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
218	09103-L31000	*CFI*	4q25	GTGTGAATGGGA-AATACATTTCTC	04-110,901
235	15918-L30940	*SLC22A5*	5q31	GAAGGAGCCCAA-CAGCACACCCAC	05-131,742
172	21193-L29851	*PKHD1*	6p12	GGAAGATTGGAA-ACTTTTGATTTT	06-052,046
500	21229-L29604	*CCDC7*	10p11	ATCGCCTTAAAC-AGAGGTCTAAAT	10-032,800
265	08938-L09033	*SLC6A5*	11p15	TTTCTGCAGGGA-TTGAATATCCTG	11-020,596
340	05388-L24168	*PKP2*	12p11	TGAGAAACTTAG-TATTTGAAGACA	12-032,895
463	21194-L29549	*MYH7*	14q11	AGGACCAGGTGA-TGCAGCAGAACC	14-022,972
310	06719-L06305	*HEXA*	15q24	GTGAGGACTTCA-AGCAGCTGGAGT	15-070,427
130	13867-L15385	*ABAT*	16p13	ACTTTGTGGAGA-AGCTCCGGCAGT	16-008,765
355	11377-L12102	*SGCA*	17q21	CTGCCCTCAACA-CCTGCCAGCCGC	17-045,601

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

Related SALSA MLPA probemixes

- **ME024 9q21 CDKN2A/2B region**: Contains probes for detection of both copy number and methylation status of genes in the 9p21 (*CDKN2A* and *CDKN2B*) region, and contains several flanking probes spread over the 9 p-arm.
- **P420 MPN mix 1**: Contains probes for the following JAK2 mutations: p.N542_E543del, p.E543_D544del and p.V617F.
- **P520 MPN mix 2**: Is a high sensitivity MLPA probemix (>1% allele burden) that contains probes for the following JAK2 mutations: p.N542_E543del, p.E543_D544del and p.V617F.

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Selected publications using SALSA MLPA Probemix P474 CD274-PDCD1LG2-JAK2

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P474 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-03 – 20 July 2022 (04P) - Product description rewritten and adapted to a new template. - Various minor textual and layout changes. - Ligation sites of the probes targeting the *CD274*, *PDCD1LG2* and *JAK2* genes updated according to new version of the NM_ reference sequences. - Warning added to Tables 1 and 2 for salt sensitivity of probes 06654-L29856 and 21210-L29585. Version A1-02 – 19 March 2019 (01P) - Length of flanking probe at 364 nt updated in Tables 1 and 2a to better represent the actual peak size. Version A1-01 – 21 September 2018 (01P) - Not applicable, new document.

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