Product Description
SALSA® MLPA® Probemix P417-B3 BAP1

To be used with the MLPA General Protocol.

Version B3

Catalogue numbers:
- **P417-025R:** SALSA MLPA Probemix P417 BAP1, 25 reactions.
- **P417-050R:** SALSA MLPA Probemix P417 BAP1, 50 reactions.
- **P417-100R:** SALSA MLPA Probemix P417 BAP1, 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 P417 BAP1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *BAP1* gene, which is associated with BAP1 tumour predisposition syndrome.

BAP1 tumour predisposition syndrome (OMIM# 614327) is inherited in an autosomal dominant manner. Individuals carrying heterozygous *BAP1* mutations are at high-risk for the development of a variety of tumours, including benign melanocytic tumours as well as several malignant tumours, including uveal melanoma, cutaneous melanoma, malignant mesothelioma upon exposure to asbestos, and other cancer types, such as renal cell carcinoma, basal cell carcinoma, lung adenocarcinoma and meningioma (Wiesner et al. 2011, Abdel-Rahman et al. 2011, Boru et al. 2019).

In addition, research suggests that uveal melanoma with monosomy of chromosome 3 (frequency 50-60% of all uveal melanomas) represents a distinct pathological entity as compared to uveal melanoma with normal disomy 3. The putative target gene on the 3p arm is *BAP1* on 3p21.1, as inactivating somatic mutations of *BAP1* are identified in >80% of patients with metastasizing uveal melanoma (Harbour et al. 2010). Moreover, *BAP1* is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma (Testa et al. 2011, Bott et al. 2011).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK390611/

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
Matched Annotation from NCBI and EMBL-EBI (MANE): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/

Exon numbering
The *BAP1* exon numbering used in this P417-B3 BAP1 product description is the exon numbering derived from MANE project (release version 1.0) based on MANE Select transcript NM_004656.4. 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. Please note that exon numbering for the same gene might be different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
The SALSA MLPA Probemix P417-B3 BAP1 contains 42 MLPA probes with amplification products between 132 and 395 nucleotides (nt). This includes 17 probes for the *BAP1* gene and 11 flanking probes included for the determination of the extent of the deletion or duplication. In addition, 14 reference probes are included that target relatively copy number stable regions in various cancer types including melanocytic tumours and mesothelioma. Complete 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)	BAP1
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-fragments (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 unrelated individuals who are from families 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 number NA04127 from the Coriell Institute and sample ID numbers SK-N-MC and ARH-77 from Leibniz Institute DSMZ have been tested with this P417-B3 probemix at MRC Holland and can be used as a positive control samples to detect copy number alterations (CNAs) as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample ID	Source	Chromosomal position of CNA*	Altered target genes in P417-B3	Expected CNA
NA04127	Coriell Institute	3p21.31-p22.2	*MLH1, RBM5, RASSF1* and *ZMYND10*	Heterozygous duplication
SK-N-MC	DSMZ	3p12.3-p22.2	*BAP1* and 3p flanking genes	Heterozygous deletion
ARH-77	DSMZ	3p12.3-p22.2	*BAP1* and 3p flanking genes	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by this P417-B3 BAP1 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 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.

P417 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, the major cause of most genetic alterations in the *BAP1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P417 BAP1.
- 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.

***BAP1* mutation databases**

We strongly encourage users to deposit positive results in the Catalogue Of Somatic Mutations In Cancer (COSMIC): http://cancer.sanger.ac.uk/cosmic; and in the Leiden Open Variation Database (LOVD): https://databases.lovd.nl/shared/genes/BAP1. 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 *BAP1* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P417-B3 BAP1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)			Location (hg18) in kb
		Reference probes	***BAP1*** [a] (3p21.1)	Flanking probes	
64-105	Control fragments – see table in probemix content section for more information				
132	Reference probe 00797-L21698	5q31			05-132,038
137	Reference probe 06972-L14818	17p13			17-006,271
142	Reference probe 10699-L11281	6p12			06-052,053
149	**BAP1 probe** 17400-L21109		**Exon 7**		03-052,416
155	Reference probe 04566-L03955	16q13			16-055,482
160	**BAP1 probe** 17401-L21110		**Exon 2**		03-052,419
166	Reference probe 12741-L21552	21q22			21-042,050
173	**BAP1 probe** 17402-L21111		**Exon 3**		03-052,419
179	**BAP1 probe** 17403-L21112		**Exon 11**		03-052,414
184 ± ¬	Flanking probe 12125-L21391			3p21.31	03-050,353
190	**BAP1 probe** 17404-L21113		**Exon 15**		03-052,412
196	Reference probe 09763-L10178	15q21			15-042,740
202 ¬	Flanking probe 22655-L31877			3p21.1	03-052,408
208	**BAP1 probe** 17405-L21114		**Exon 5**		03-052,417
214 ¬	Flanking probe 03206-L13082			3p21.31	03-050,358
221 ¬	Flanking probe 07223-L21127			3p14.3	03-057,207
228	**BAP1 probe** 16643-L19177		**Exon 4**		03-052,418
232	**BAP1 probe** 17406-L21392		**Exon 1**		03-052,419
238	Reference probe 19768-L31764	12q12			12-041,074
244	**BAP1 probe** 17407-L21116		**Exon 13**		03-052,413
251	Reference probe 08811-L31763	2p13			02-071,620
257	**BAP1 probe** 17408-L21394		**Exon 6**		03-052,416
263	**BAP1 probe** 21243-L29765		**Exon 14**		03-052,412
270	**BAP1 probe** 17398-L32045		**Exon 9**		03-052,415
276 ¬	Flanking probe 06439-L32044			3p12.3	03-079,071
282	**BAP1 probe** 21244-L32082		**Exon 16**		03-052,412
287	**BAP1 probe** 17411-L32043		**Exon 10**		03-052,415
293	Reference probe 08936-L32083	11p15			11-020,586
299 ±	**BAP1 probe** 17412-L21397		**Exon 8**		03-052,416
308	**BAP1 probe** 17413-L21122		**Exon 12**		03-052,414
317 ¬	Flanking probe 04710-L01787			3p14.2	03-059,883
325	Reference probe 16275-L21395	19p13			19-012,782
332	**BAP1 probe** 17414-L21123		**Exon 17**		03-052,411
338	Reference probe 12785-L15496	2q13			02-108,972
346 ¬	Flanking probe 10794-L11434			3p14.1	03-070,081
352 ¬	Flanking probe 22656-L31878			3p21.1	03-052,430

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)			Location (hg18) in kb
		Reference probes	***BAP1*** [a] (3p21.1)	Flanking probes	
359	Reference probe 11614-L31880	12p13			12-004,894
364 ¬	Flanking probe 15897-L18094			3p21.31	03-050,105
372	Reference probe 06016-L21128	19q13			19-059,319
378 ¬	Flanking probe 14836-L21403			3**q**11.2	03-099,792
385 ¬	Flanking probe 16176-L21699			3p22.2	03-037,057
395	Reference probe 05914-L21838	18p11			18-013,724

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

± SNPs rs587745848 and rs202170860 could influence the probe signal of the 184 nt and 299 nt probes respectively. In case of apparent deletions, it is recommended to sequence the region targeted by the corresponding probe.

¬ 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. P417-B3 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	Location (hg18) in kb
385 ¬	16176-L21699	*MLH1*	3p22.2	CGTGGGCTGTGT-GAATCCTCAGTG	13.1 **M**b	03-037,057
364 ¬	15897-L18094	*RBM5*	3p21.31	ATATGATGACTA-CCGAGACTATGA	248.6 kb	03-050,105
184 ± ¬	12125-L21391	*RASSF1*	3p21.31	CAAAGCCAGCGA-AGCACGGGCCCA	4.9 kb	03-050,353
214 ¬	03206-L13082	*ZMYND10*	3p21.31	CCGCACTGCGCA-GGCGCGGCTAAC	2.1 **M**b	03-050,358
202 ¬	22655-L31877	*DNAH1*	3p21.1	CTTGAGTGCCTT-GAGTAGGTCTTG	3.2 kb	03-052,408
			Telomeric			
BAP1, at 3p21.1. Ligation sites are indicated according to MANE Select transcript NM_004656.4						
		stop codon	2318-2320			
332	17414-L21123	***BAP1***, ex 17	2483-2484	TCCATCGTGCCC-TGAGGCCTGACA	0.6 kb	03-052,411
282	21244-L32082	***BAP1***, ex 16	2117-2118	TCCTACAGATTG-ATGACCAGAGAA	0.2 kb	03-052,412
190	17404-L21113	***BAP1***, ex 15	2042-2043	CACTGCTGAAGT-GTGTGGAGGCTG	0.3 kb	03-052,412
263	21243-L29765	***BAP1***, ex 14	2014-2015	GAGAAATACTCA-CCCAAGGTGAGC	0.4 kb	03-052,412
244	17407-L21116	***BAP1***, ex 13	1717-1718	CACATCTCCAAG-GTGCTTTTTGGA	1.0 kb	03-052,413
308	17413-L21122	***BAP1***, ex 12	1291-1292	AGCCGAGTTCCA-GTCCGCCCACCC	0.6 kb	03-052,414
179	17403-L21112	***BAP1***, ex 11	1245-1246	GTCCCCCATGCA-GGTAAGCTGGGA	0.8 kb	03-052,414
287	17411-L32043	***BAP1***, ex 10	960-961	CCACAAGTCTCA-AGAGTCACAGCT	0.5 kb	03-052,415
270	17398-L32045	***BAP1***, ex 9	826-827	AACCTGATGGCA-GTGGTGCCCGAC	0.5 kb	03-052,415
299 ±	17412-L21397	***BAP1***, ex 8	782-783	TCGGCCTCGCCA-CTGCAGGGTAAG	0.4 kb	03-052,416
149	17400-L21109	***BAP1***, ex 7	613-614	GGCCTTAGTGCA-GTGCGGACCATG	0.2 kb	03-052,416
257	17408-L21394	***BAP1***, ex 6	513-514	GTAGAGCAAAGG-ATATGCGATTGG	0.6 kb	03-052,416
208	17405-L21114	***BAP1***, ex 5	386-387	TGGTTTCACAGC-TGATACCCAACT	0.4 kb	03-052,417
228	16643-L19177	***BAP1***, ex 4	344-345	ATACGTCCGTGA-TTGATGATGATA	1.1 kb	03-052,418
173	17402-L21111	***BAP1***, ex 3	224-225	AAGTGGAGGAGA-TCTACGACCTTC	0.2 kb	03-052,419
160	17401-L21110	***BAP1***, ex 2	187-186 reverse	ACCGAAATCTTC-CACGAGCAGGGT	0.1 kb	03-052,419
232	17406-L21392	***BAP1***, ex 1	141-140 reverse	GCTCCAGCCAGC-CCTTATTCATCT	11.1 kb	03-052,419
		start codon	131-133			
			Centromeric			
352 ¬	22656-L31878	*PHF7*	3p21.1	CAACAGAGTGTT-GAGAACATCCAG	4.8 **M**b	03-052,430

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
221 ¬	07223-L21127	*HESX1*	3p14.3	ATCGATATTAGA-GAAGACTTAGCT	2.7 **M**b	03-057,207
317 ¬	04710-L01787	*FHIT*	3p14.2	TCTCCAGCCTTC-CTGGGAAGAACA	10.2 **M**b	03-059,883
346 ¬	10794-L11434	*MITF*	3p14.1	TCTTTATGGAAA-CCAAGGTCTGCC	9.0 **M**b	03-070,081
276 ¬	06439-L32044	*ROBO1*	3p12.3	CCACCTCGCATT-GTTGAACACCCT	20.7 **M**b	03-079,071
			Centromere			
378 ¬	14836-L21403	*CPOX*	3**q**11.2	GTGCTATGGGCG-TGAGCTCTGTTA	-	03-099,792

[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.

± SNPs rs587745848 and rs202170860 could influence the probe signal of the 184 nt and 299 nt probes respectively. In case of apparent deletions, it is recommended to sequence the region targeted by the corresponding probe.

¬ 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 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
251	08811-L31763	*DYSF*	2p13	TACCACCTACCT-GAGTATGTCGAA	02-071,620
338	12785-L15496	*EDAR*	2q13	CCCAGAACTGGA-TGGTACCTGACT	02-108,972
132	00797-L21698	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132,038
142	10699-L11281	*PKHD1*	6p12	AGGGTCTGTACT-TCCTGGAAGCAT	06-052,053
293	08936-L32083	*SLC6A5*	11p15	TGTTTGCCTCCT-TTGTGTCTGTAC	11-020,586
359	11614-L31880	*KCNA1*	12p13	AACTAAACCAAT-TGATTTAATAGT	12-004,894
238	19768-L31764	*PPHLN1*	12q12	CAAGTGGGCTGC-TGAAAAGCTAGA	12-041,074
196	09763-L10178	*SPG11*	15q21	AACCATTTTGTA-TAGCTGTAGTAG	15-042,740
155	04566-L03955	*SLC12A3*	16q13	TGTGTCATGAGG-ATGCGGGAGGGA	16-055,482
137	06972-L14818	*AIPL1*	17p13	ATCGGCTCTTCA-AGCTGGGCCGCT	17-006,271
395	05914-L21838	*RNMT*	18p11	TACAATGAACTT-CAGGAAGTTGGT	18-013,724
325	16275-L21395	*RNASEH2A*	19p13	GGTCAAGGCCAA-AGCAGATGCCCT	19-012,782
372	06016-L21128	*PRPF31*	19q13	ACAAGTGCAAGA-ACAATGAGAACC	19-059,319
166	12741-L21552	*RIPK4*	21q22	AAGCCAAGAAGA-TGGAGATGGCCA	21-042,050

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

Related SALSA MLPA probemixes

- **P027 Uveal melanoma** Contains probes for chromosomes 1p, 3, 6 and 8.
- **P226 SDH** Contains probes for all exons of the *SDHB, SDHC, SDHD, SDHAF1* and *SDHAF2* genes.
- **P419 CDKN2A/2B-CDK4** Contains probes for the *CDKN2A/2B* and *CDK4* genes and flanking probes on chromosome 9.
- **P429 SDHA-MAX-TMEM127** Contains probes for most exons (10 of 15 exons) of the *SDHA* gene and all exons of the *MAX* gene.

- **ME024 9p21 CDKN2A/2B region** Contains probes for the CDKN2A/2B region at 9p21.3 and for the surrounding genes, both for copy number and methylation status determination.

References

- Abdel-Rahman MH et al. (2011). Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. *J Med Genet*. 48:856-9.
- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol*. 147:60-8.
- Boru G et al. (2019). Germline large deletion of BAP1 and decreased expression in non-tumor choroid in uveal melanoma patients with high risk for inherited cancer. *Genes Chromosomes Cancer*. 58:650-6.
- Bott M et al. (2011). The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat Genet*. 43:668-72.
- Harbour JW et al. (2010). Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science*. 330:1410-3.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- 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.
- Testa JR et al. (2011). Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet. 43:1022-5.
- 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.
- Wiesner T et al. (2011). Germline mutations in BAP1 predispose to melanocytic tumors. *Nat Genet*. 43:1018-21.

Selected publications using SALSA MLPA Probemix P417 BAP1

- Abbassi YA et al. (2023). Analysis of uveal melanomas and paired constitutional DNA for exclusion of a BAP1-tumor predisposition syndrome. *Fam Cancer*. 22:193-202.
- Betti M et al. (2015). Inference on germline BAP1 mutations and asbestos exposure from the analysis of familial and sporadic mesothelioma in a high-risk area. *Genes Chromosomes Cancer*. 54:51-62.
- Betti M et al. (2018). Sensitivity to asbestos is increased in patients with mesothelioma and pathogenic germline variants in BAP1 or other DNA repair genes. *Genes Chromosomes Cancer*. 57:573-83.
- Boru G et al. (2019). Germline large deletion of BAP1 and decreased expression in non-tumor choroid in uveal melanoma patients with high risk for inherited cancer. *Genes Chromosomes Cancer*. 58:650-6.
- Cheung M et al. (2015). An asbestos-exposed family with multiple cases of pleural malignant mesothelioma without inheritance of a predisposing BAP1 mutation. *Cancer Genet*. 208:502-7.
- Mori T et al. (2015). Somatic alteration and depleted nuclear expression of BAP1 in human esophageal squamous cell carcinoma. *Cancer Sci*. 106:1118-29.
- Nasu M et al. (2015). High Incidence of Somatic BAP1 Alterations in Sporadic Malignant Mesothelioma. *J Thorac Oncol*. 10:565-76.
- Rai K et al. (2016). Germline alterations in familial uveal melanoma. *Genes Chromosomes Cancer*. 56:168-74.
- Repo P et al. (2019). Population-based analysis of BAP1 germline variations in patients with uveal melanoma. *Hum Mol Genet*. 28:2415-26.
- de Reyniès A et al. (2014). Molecular classification of malignant pleural mesothelioma: identification of a poor prognosis subgroup linked to the epithelial-to-mesenchymal transition. *Clin Cancer Res*. 20:1323-34.

- Sculco M et al. (2022). Diagnostics of BAP1-Tumor Predisposition Syndrome by a Multitesting Approach: A Ten-Year-Long Experience. *Diagnostics (Basel)*. 12.
- Van de Nes JA et al. (2016). Comparing the prognostic value of BAP1 mutation pattern, chromosome 3 status, and BAP1 Immunohistochemistry in Uveal Melanoma. *Am J Surg Pathol*. 40:796-805.

P417 product history	
Version	*Modification*
B3	Two flanking probes and two reference probes have been replaced and one reference probe is removed. Additionally, several probes have a change in length but no change in the sequences detected.
B2	Two reference probes have been added and two target probes have a minor change in length.
B1	First unrestricted release. New probes for BAP1 gene have been included for exons that were not covered in the previous version. In addition, all reference probes have been replaced.
A1	First restricted release.

Implemented changes in the product description

Version B3-02 – 28 February 2024 (04P)
- Product description rewritten and adapted to a new template.
- Warning added to Tables 1 and 2 for SNPs that could affect probe signal.
- New references added in 'Selected publications using SALSA MLPA Probemix P417 BAP1' section on page 8.

Version B3-01 — 26 March 2020 (02P)
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2a/b) and adapted to a new template.
- Ligation sites of the probes targeting the BAP1 gene updated according to new version of the NM_ reference sequence.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- Related probemixes section updated on page 7.
- Selected publications list updated on page 7-8.

Version 07 – 24 April 2019 (T08)
- New references added on page 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Minor textual changes throughout the document.

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