

Product Description

SALSA® MLPA® Probemix P370-BRAF-IDH1-IDH2

To be used with the MLPA General Protocol.

Version C1

For complete product history see page 12.

Catalogue numbers

- **P370-025R:** SALSA® MLPA® Probemix P370 BRAF-IDH1-IDH2, 25 reactions
- **P370-050R:** SALSA® MLPA® Probemix P370 BRAF-IDH1-IDH2, 50 reactions
- **P370-100R:** SALSA® MLPA® Probemix P370 BRAF-IDH1-IDH2, 100 reactions

SALSA® MLPA® Probemix P370 BRAF-IDH1-IDH2 (hereafter: P370 BRAF-IDH1-IDH2) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

P370 BRAF-IDH1-IDH2 can be used in combination with:

- SALSA® Binning DNA SD054 (Cat. No: SD054)

Volumes and ingredients

Volumes			Ingredients
P370-025R	P370-050R	P370-100R	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		
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A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis

Information regarding 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

SALSA® MLPA® Probemix P370 BRAF-IDH1-IDH2 is a **research use only (RUO)** assay for the detection of the *BRAF* p.V600E & four predominant *IDH1* (p.R132H and p.R132C) and *IDH2* (p.R172M and p.R172K) point mutations, for detection of genomic duplications leading to the *SRGAP3-RAF1*, *KIAA1549-BRAF* and *FGFR1-TACC1* fusion genes on chromosome arms 3p, 7q and 8p respectively, and for detection of copy number aberrations in the *BRAF*, *CDKN2A/2B*, *FGFR1*, *MYB* and *MYBL1* genes.

The *IDH1* and *IDH2* mutations represent frequent genetic abnormalities in gliomas. Their identification facilitates distinguishing the different glioma entities leading to a more accurate prognosis and treatment (Riemenschneider et al. 2010). *IDH1* and *IDH2* point mutations have been detected with high frequency in diffuse gliomas (Hartmann et al. 2009; Yan et al. 2009), and the presence of these mutations is associated with a longer survival (Sanson et al. 2009; van den Bent et al. 2010). *IDH1/2* mutation is a marker for glioma classification since 2016, defining glioblastomas as *IDH*-mutant or *IDH*-wildtype (Wesseling and Capper 2018). This MLPA probemix contains probes that are specific for the two most frequent *IDH1* (p.R132H and p.R132C) and the two most frequent *IDH2* (p.R172M and p.R172K) mutations.

Activation of the *MAPK* pathway has been detected with high frequency in pilocytic astrocytomas, in particular via a 2 Mb tandem duplication leading to an oncogenic *KIAA1549-BRAF* fusion gene at 7q34 (Jones et al. 2008). Detection of this duplication is of help in differentiating these tumours from diffuse astrocytomas. Alternative *MAPK* pathway activation mechanisms include: 1) the formation of a similar *SRGAP3-RAF1* fusion gene at 3p25, through a 3.6 Mb tandem duplication (Jones et al. 2009), 2) intragenic duplications of *FGFR1* and *FGFR1-TACC1* microamplifications (Zhang et al. 2013; Jones et al. 2013), and 3) certain *BRAF* mutations, in particular the p.V600E mutation (Schiffman et al. 2010; Dougherty et al. 2010). The *BRAF* p.V600E activating mutation in combination with deletion of *CDKN2A* was found to be significantly enriched in cases of low grade glioma that are undergoing transformation to secondary high grade gliomas (Mistry et al. 2015), suggesting to define a clinical distinct subgroup of childhood glioma.

This probemix also includes probes for the *FGFR1*, *MYB* and *MYBL1* genes and for the 9p21.3 region (*CDKN2A/2B*, *MIR31*). All these genes and regions are suggested to help in differentiating molecular subtypes of gliomas (see Table 2 for more detailed information). Furthermore, this probemix contains 13 reference probes detecting autosomal chromosomal locations that are regarded as relatively stable in gliomas and brain tumours. However, it should be noticed that glioma karyotypes can harbour multiple numerical and structural aberrations, which can complicate interpretation of these reference probes.

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:

Entrez Gene shows transcript variants of each gene: <https://www.ncbi.nlm.nih.gov/gene>

For NM_ mRNA reference sequences: <https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide>

Matched Annotation from NCBI and EMBL-EBI (MANE): <https://www.ncbi.nlm.nih.gov/refseq/MANE>

Locus Reference Genomic (LRG) database: <http://www.lrg-sequence.org/>

Exon numbering

The exon numbering used in this P370-C1 BRAF-IDH1-IDH2 product description is the exon numbering from MANE project (release version 1.5) based on MANE Select transcripts NM_005896.4 for *IDH1*, NM_014850.4 for *SRGAP3*, NM_002880.4 for *RAF1*, NM_001130173.2 for *MYB*, NM_001164665.2 for *KIAA1549*, NM_004333.6 for *BRAF*, NM_023110.3 for *FGFR1*, NM_006283.3 for *TACC1*, NM_001080416.4 for *MYBL1* and NM_002168.4 for *IDH2*. The exon numbering for *CDKN2A* is based on MANE Select transcript NM_000077.5 encoding p16INK4A and MANE Plus Clinical transcript NM_058195.4 encoding p14ARF. Both NM_000077.5 and NM_058195.4 have distinct first exons (both numbered as exon 1) which contain the translation start codon, and share a common second exon, which is translated in different reading frames. The *IDH1*, *SRGAP3*, *TACC1*, *IDH2* exon numbering has changed; the exon numbering used in previous versions of this product

description can be found in between brackets in Table 2. 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

P370-C1 BRAF-IDH1-IDH2 contains 59 MLPA probes with amplification products between 124 and 500 nucleotides (nt). This includes 41 probes for the detection of genomic duplications leading to the *KIAA1549-BRAF*, *SRGAP3-RAF1* and *FGFR1-TACC1* fusion genes, and for the detection of copy number aberrations in the *BRAF*, *CDKN2A/2B*, *FGFR1*, *MYB* and *MYBL1* genes. Furthermore, this probemix also contains five probes specific for the *BRAF* p.V600E mutation & four predominant *IDH1* p.R132H and p.R132C and *IDH2* p.R172M and p.R172K point mutations, which will only generate a signal when the mutation is present. In addition, 13 reference probes are included that target relatively copy number stable regions in various cancer types including brain tumours. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com) and in Table 3.

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 MLPA (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 using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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. Note that the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.

Required specimens

Extracted DNA, which includes DNA derived from formalin-fixed, paraffin-embedded (FFPE) tissues, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from healthy individuals without a history of brain tumours. 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

See the section “Positive samples” on the P370 product page on our website.

SALSA® Binning DNA SD054

The SALSA® Binning DNA SD054 provided with this probemix can be used for binning of all probes including the five mutation-specific probes (226 nt probe M08780-SP0039-L08904 for *BRAF* p.V600E, 203 nt probe M19529-L16492 for *IDH1* p.R132H, 220 nt probe M14787-L16493 for *IDH1* p.R132C, 244 nt probe M20963-L29001 for *IDH2* p.R172M, and 238 nt probe M20963-L29002 for *IDH2* p.R172K). SD054 is a mixture of human female genomic DNA from healthy individuals and a titrated amount of plasmid DNA that contains the target sequence detected by the above mentioned probes. Inclusion of one reaction with 5 μ l SD054 in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net. Furthermore, binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signals. For further details, please consult the SD054 product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 (with exception of the mutation-specific probes) 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/gain	$1.30 < FR < 1.65$
Heterozygous triplication/homozygous duplication/gain	$1.75 < FR < 2.15$
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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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.

The above mentioned FR values do not apply to the mutation-specific probes. The peaks of the mutation-specific probes are expected to be absent in the majority of samples tested and therefore their standard deviation cannot be determined. Clear signal (at least 10% of the median peak height of all reference probes in that sample) for one of these probes indicates that the mutation is present.

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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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: <https://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.

P370 BRAF-IDH1-IDH2 specific note(s):

- The presence of a clear signal for the 226, 203, 220, 244 and 238 probes (at least 10% of the mean peak height of all reference probes in the sample), indicates the presence of the *BRAF* p.V600E, *IDH1* p.R132H, *IDH1* p.R132C, *IDH2* p.R172M and *IDH2* p.R172K mutation, respectively. The mutation-specific probes are only intended to determine the presence (or absence) of the mutations but not for their quantification.
- Use of FFPE tissues can result in low quality of the extracted DNA due to sample fixation and storage conditions. This might result in higher probe standard deviations. Warnings during the Fragment Analysis using Coffalyser.Net will indicate that the MLPA experiment was not optimal on the specific sample(s) used. For more information on the use of FFPE tissues with MLPA, please refer to Atanesyan et al. 2017.
- Please note that due to high nucleotide sequence similarity of mutated V600E (GTG to GAG single nucleotide variation) and V600K (GTG to AAG double nucleotide variation) codons, the *BRAF* V600E probe included in this probemix might give a small signal on a sample with the V600K mutation.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *BRAF*, *IDH1*, *IDH2*, *KIAA1549*, *SRGAP3*, *RAF1*, *FGFR1*, *TACC1*, *CDKN2A/2B*, *MYB* and *MYBL1* genes are small (point) mutations, most of which will not be detected by using P370 BRAF-IDH1-IDH2. The *BRAF* p.V600E, *IDH1* p.R132H, *IDH1* p.R132C, *IDH2* p.R172M and *IDH2* p.R172K mutations can be detected, but other point mutations in the target genes cannot be detected.
- 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 brain tumours with more chaotic karyotypes.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. Copy number changes detected by only a single probe as well as point mutations always require confirmation by another method. Because the mutation-specific probes are only intended to determine the presence of the mutation, positive results obtained for either of these probes need to be confirmed by sequence analysis to determine the zygosity of the mutation. 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 in sequence data 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

<https://cancer.sanger.ac.uk/cosmic>. We strongly encourage users to deposit positive results in the COSMIC mutation database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on <https://varnomen.hgvs.org>.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *BRAF* exons 8 and 14 but not exon 12) to MRC Holland: info@mrcholland.com.

Table 1. P370-C1 BRAF-IDH1-IDH2

Length (nt)	MLPA probe	Chromosomal position (hg18)						
		Reference	IDH1/2	3p	6q	Chr. 7	Chr. 8	9p
64-105	Control fragments – see table in probemix content section for more information							
124	Reference probe 18709-L21056	5q31						
130 *	Reference probe 19551-L31871	2p13						
135 ¥	MYBL1 probe 22553-L31748						8q13.1	
139 ¥	SRGAP3 probe 19365-L31872			3p25.3				
143 ¥	Reference probe 16316-L31755	3q21						
148 «	MYB probe 12500-L25742				6q23.3			
155 ~	LAMA2 probe 14924-L25757				6q22.33			
160	SRGAP3 probe 19366-L25759			3p25.3				
166	KIAA1549 probe 15251-L17567					7q34		
172	MYBL1 probe 07915-L07628						8q13.1	
179	BRAF probe 10509-L25743					7q34		
184	FGFR1 probe 04184-L25753						8p12	
190	TACC1 probe 19368-L25761						8p11.23	
196	Reference probe 18049-L22439	16q23						
203 §	IDH1 probe 19529-L16492		p.R132H					
208	KIAA1549 probe 15252-L17007					7q34		
214 ¥ #	BRAF probe 22554-L26031					7q34		
220 §	IDH1 probe 14787-L16493		p.R132C					
226 § ¥ §	BRAF probe 08780-SP0039-L08904						p.V600E	
232	Reference probe 16428-L25931	18q21						
238 ¥ §	IDH2 probe 20963-L29002		p.R172K					
244 ¥ §	IDH2 probe 20963-L29001		p.R172M					
249	CDKN2A probe 10333-L17690							9p21.3
254	RAF1 probe 15332-L17816			3p25.1				
261	MYBL1 probe 07914-L26455						8q13.1	
266	KIAA1549 probe 15253-L17561					7q34		
274 ~	CNTNAP2 probe 12947-L25756					7q35		
280	Reference probe 13350-L26120	9q21						
285	CDKN2A probe 16533-L26121							9p21.3
292 ~ «	IKZF1 probe 16911-L15654					7p12.2		
298	MYB probe 17265-L26123				6q23.3			
304 ~	SLC26A3 probe 17066-L26124					7q22.3		
310	Reference probe 16559-L26125	11q13						
319	KIAA1549 probe 15255-L17010					7q34		
326	MYBL1 probe 19605-L26457						8q13.1	
333 ~	PLAGL1 probe 18472-L26458				6q24.2			
339	FGFR1 probe 17635-L26228						8p12	
346 *	Reference probe 05273-L04655	2p22						
353	FGFR1 probe 04439-L26460						8p12	
360	MKRN1 probe 15257-L26461					7q34		
366 *	RAF1 probe 22758-L32102			3p25.1				
373	FGFR1 probe 18296-L25750						8p12	
382 *	Reference probe 09717-L31870	12q24						
388	MIR31 probe 19508-L26462							9p21.3
395	HIPK2 probe 15259-L26463					7q34		
402	FGFR1 probe 04440-L26464						8p12	
409 #	BRAF probe 19324-L25551					7q34		
417	Reference probe 13817-L15311	2q13						
424	FGFR1 probe 04441-L21311						8p12	
430	MYB probe 19369-L26102				6q23.3			
438	TACC1 probe 19370-L25763						8p11.23	
447	BRAF probe 19328-L25555					7q34		
454	CDKN2B probe 01531-L13742							9p21.3
463 *	Reference probe 10685-L31869	6p12						

Length (nt)	MLPA probe	Chromosomal position (hg18)						
		Reference	IDH1/2	3p	6q	Chr. 7	Chr. 8	9p
471	MYB probe 19371-L26746				6q23.3			
478 ¥	BRAF probe 21619-L31757					7q34		
484 ~	CRBN probe 06312-L26467			3p26.3				
494	Reference probe 19137-L26747	21q22						
500 ¥	Reference probe 19675-L27812	4p13						

* New in version C1.

¥ Changed in version C1. Minor alteration, no change in sequence detected.

§ Mutation-specific probe. This probe will only generate a signal when the *BRAF* p.V600E, *IDH1* p.R132H / p.R132C or *IDH2* p.R172M / p.R172K mutation is present.

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

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

~ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene, pseudogene or highly similar region.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. Target and flanking probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene (exon) ^a	Location (hg18) / Ligation site ^b	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
IDH1 p.R132H and p.R132C, at 2q33.3					
These probes will only give a signal when either the p.R132H or the p.R132C mutation is present in the sample. The p.R132H (c.395G>A) mutation has been detected by sequencing in 664 samples covering 92.7% of <i>IDH1</i> mutations, and the p.R132C (c.394C>T) mutation in 29 samples covering 4.2% of the <i>IDH1</i> mutations in a cohort of 1010 diffuse gliomas (Hartmann et al. 2009). No probes are present in this probemix for the <i>IDH1</i> p.R132S (c.394C>A), p.R132G (c.394C>G) and p.R132L (c.395G>T) mutations which were detected in 11 (1.5%), 10 (1.5%) and two (0.2%) samples, respectively, in the same study by Hartmann et al. 2009.					
203 §	19529-L16492	<i>IDH1</i> , ex 4 (6) c.395G>A=p.R132H	NM_005896.4; 618-619	CATCATAGGTCA-TCATGCTTATGG	0.0 kb
220 §	14787-L16493	<i>IDH1</i> , ex 4 (6) c.394C>T=p.R132C	NM_005896.4; 617-616 reverse	ATAAGCATGACA-ACCTATGATGAT	-
SRGAP3-RAF1 fusion, at 3p25					
Activation of the <i>RAF1</i> gene by formation of a <i>SRGAP3-RAF1</i> fusion gene in a similar way as the <i>KIAA1549-BRAF</i> fusion gene has been described in pilocytic astrocytoma patients (Jones et al. 2009; Forshew et al. 2009; Tatevossian et al. 2010). It is indicated in the 4 th column whether the probe is located within or outside the duplicated region.					
484 ~	06312-L26467	<i>CRBN</i>	3p26.3; <i>outside</i>	CCGATGGCAGCT-TATGTGAATCCT	5.8 Mb
160	19366-L25759	<i>SRGAP3</i> , ex 19 (20)	NM_014850.4; 2933-2934; <i>outside</i>	GAAGTGGAGCAG-ATCGAGGCTATT	130.4 kb
139	19365-L31872	<i>SRGAP3</i> , ex 2	NM_014850.4; 812-813; <i>within</i>	GAGCAGCAATCA-GAGTCGCGACTG	3.5 Mb
254	15332-L17816	<i>RAF1</i> , ex 17	NM_002880.4; 2949-2950; <i>within</i>	TCATGCTGAATT-TTGTCTTCAGG	34.7 kb
366	22758-L32102	<i>RAF1</i> , ex 2	NM_002880.4; 477-478; <i>outside</i>	TGGCAAACAC-AGATCCTTCTAA	11.7 kb
MYB aberrations, at 6q23.3					
MYB is an oncogenic transcription factor that can act both as a transcriptional activator and repressor. Both amplifications of the whole <i>MYB</i> gene and 3' deletions of the <i>MYB</i> gene have been described in paediatric low-grade gliomas (Tatevossian et al. 2010; Zhang et al. 2013).					
155 ~	14924-L25757	<i>LAMA2</i>	6q22.33	GGGTTTCAAACA-GATGTCAGAGTT	6.0 Mb
148 «	12500-L25742	<i>MYB</i> , ex 2	NM_001130173.2; 265-266	TGAGGACTTTGA-GATGTGTGACCA	11.2 kb
471	19371-L26746	<i>MYB</i> , ex 10	NM_001130173.2; 1604-1605	CCACCCAAGGTC-TTACCTCCTGCA	6.1 kb
430	19369-L26102	<i>MYB</i> , ex 15	NM_001130173.2; 2285-2286	CTTACAAGCTCC-GTTTAAATGGCA	15.2 kb
298	17265-L26123	<i>MYB</i> , ex 16	NM_001130173.2; 2969-2970	CATTTAATCCAG-ATTGTAATGCT	8.7 Mb
333 ~	18472-L26458	<i>PLAGL1</i>	6q24.2	AAGTTTGCTGA-AGATTCAAACCT	-

Length (nt)	MLPA probe	Gene (exon) ^a	Location (hg18) / Ligation site ^b	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
KIAA1549-BRAF fusion and BRAF p.V600E mutation, at 7q34 MAPK pathway activation through a 2 Mb tandem duplication leading to an oncogenic KIAA1549-BRAF fusion gene is suggested to be a very frequent event in pilocytic astrocytomas (Jones et al. 2008). The BRAF p.V600E mutation, which can be detected by the presence of the 226 nt probe, is also found regularly in this type of tumours. It is indicated in the 4 th column whether the probe is located within or outside the commonly duplicated region. Note that several different fusion variants have been described in the literature. The most common is the KIAA1549 exon 16-BRAF exon 9 fusion. For more information on other fusion variants see e.g. Table 1 in Forsheew et al. 2009. When the BRAF p.V600E point mutation is detected in combination with deletion of CDKN2A, paediatric low grade glioma patients have an increased risk for transformation to secondary high grade glioma; therefore, therapy may need other preventive measures for this defined clinical distinct subgroup (Mistry et al. 2015).					
7p arm					
292 ~ «	16911-L15654	IKZF1	7p12.2; <i>outside</i>	AGACATGTCCCA-AGTTTCAGGTGA	56.9 Mb
7q arm					
304 ~	17066-L26124	SLC26A3	7q22.3; <i>outside</i>	CCACTTCTGCA-TGTGGCAGAAAG	31.0 Mb
166	15251-L17567	KIAA1549, ex 19	NM_001164665.2; 5617-5618; <i>outside</i>	TTGCCAGCAGAA-TTGAGCTCAGC	12.0 kb
266	15253-L17561	KIAA1549, ex 17	NM_001164665.2; 5387-5388; <i>outside</i>	AGACTATGGAAT-GACTCCCCGAC	29.3 kb
208	15252-L17007	KIAA1549, ex 11	NM_001164665.2; 4212-4213; <i>within</i>	GGTCAGCACAAT-AAAGACGACATA	29.7 kb
319	15255-L17010	KIAA1549, ex 4	NM_001164665.2; 3206-3207; <i>within</i>	CCAGACTCCTTT-AATCCTGTCTGT	703.2 kb
395	15259-L26463	HIPK2	7q34; <i>within</i>	ACTACCATCTA-CACTCTACCAGC	855.8 kb
360	15257-L26461	MKRN1	7q34; <i>within</i>	ACCACCTCTTA-CACATTTTCAGC	280.6 kb
179	10509-L25743	BRAF, ex 17	NM_004333.6; 8 nt after exon 17; <i>within</i>	CCAAGTAAGTAA-AAGCTTCATGCT	13.5 kb
226 § Ж §	08780-SP0039-L08904	BRAF, ex 15 p.V600E=c.1799T>A	NM_004333.6; 1985-1986 and 2025-2026	TTCTTCATGAAG-ACCTCACAGTAAA AATAGGTGATTTTGGTCTAGCTACAGA- GAAATCTCGATG	0.9 kb
447	19328-L25555	BRAF, ex 14	NM_004333.6; 1964-1965; <i>within</i>	ACCTCAAGAGTA-ATAGTATCCTTC	23.9 kb
409 #	19324-L25551	BRAF, ex 12	NM_004333.6; 1688-1689; <i>within</i>	TGTTGAATGTGA-CAGCACCTACAC	16.4 kb
478	21619-L31757	BRAF, ex 8	NM_004333.6; 1215-1216; <i>outside</i>	CAGGCCCAAAT-TCTCACCAGTCC	14.5 kb
214 #	22554-L26031	BRAF, ex 4	NM_004333.6; 758-759; <i>outside</i>	GAGTTACAGTCC-GAGACAGTCTAA	6.2 Mb
274 ~	12947-L25756	CNTNAP2	7q35; <i>outside</i>	GTGCTCTGGAT-TGGAATGGAGAA	-
Intragenic duplications of FGFR1 and FGFR1-TACC1 microamplifications, at 8p11-p12 Intragenic duplications of exons 10-18 or exons 11-18 of the FGFR1 gene are detected both in paediatric low grade diffuse gliomas and in pilocytic astrocytomas (Zhang et al. 2013; Jones et al. 2013). These intragenic duplications are suggested to produce autophosphorylation of FGFR1 and upregulation of the MAPK/ERK and PI3K pathways. In addition, microamplifications of FGFR1 and TACC1 leading to in-frame FGFR1-TACC1 fusions by joining exon 18 of FGFR1 with exon 7 of TACC1, have been described in low-grade gliomas (Zhang et al. 2013).					
424	04441-L21311	FGFR1, ex 18	NM_023110.3; 3530-3531	AGCCAATGAACA-GGCATGCAAGTG	1.6 kb
402	04440-L26464	FGFR1, ex 14	NM_023110.3; 2609-2610	TGCATACACCGA-GACCTGGCAGCC	1.1 kb
353	04439-L26460	FGFR1, ex 13	NM_023110.3; 2511-2512	ACCCAGCCACA-ACCCAGAGGAGC	2.4 kb
373	18296-L25750	FGFR1, ex 10	NM_023110.3; 2060-2061	TCCATGAACTCT-GGGGTTCTTCTG	9.7 kb
184	04184-L25753	FGFR1, ex 5	NM_023110.3; 1282-1283	CAAATGCCCTTC-CAGTGGGACCCC	29.4 kb
339	17635-L26228	FGFR1, ex 2	NM_023110.3; 756-757	TGTGGAGCTGGA-AGTGCTCTCTCT	271.0 kb
190	19368-L25761	TACC1, upstream (ex 1)	NM_006283.3; 58.8 kb before exon 1	GCTGACTTCGCA-CTTGAGCTCCAG	113.9 kb
438	19370-L25763	TACC1, ex 10 (11)	NM_006283.3; 2372-2373	ATGGAGAAGGAA-CAGGCCCTGGCT	28.8 Mb
Duplications of MYBL1, at 8q13.1 MYBL1 belongs to the Myb family of transcription factors. Gain of 8q13.1 is detected in 28% of paediatric diffuse astrocytomas resulting in partial duplication of MYBL1 with truncation of its C-terminal negative-regulatory domain (Ramkissoon et al. 2013; Zhang et al. 2013).					
172	07915-L07628	MYBL1, ex 14	NM_001080416.4; 2312-2313	CTGTTGACTGAA-GACATTTTCAGAC	25.8 kb
326	19605-L26457	MYBL1, ex 8	NM_001080416.4; 1227-1228	AGAATGAAGTTA-GAAGAAAGCGAA	4.9 kb
135	22553-L31748	MYBL1, ex 5	NM_001080416.4; 836-837	ATCATCTATGAA-GCACAATAAGCGG	5.1 kb
261	07914-L26455	MYBL1, ex 2	NM_001080416.4; 443-444	GATCATGATTAT-GAAGTACCACAA	-
CDKN2A, CDKN2B and MIR31 genes, at 9p21.3 Loss of 9p, and especially deletions of the 9p21.3 region including CDKN2A, are common in high-grade gliomas. In contrast, CDKN2A deletions are rare in anaplastic astrocytomas and glioblastomas with mutated IDH1 or IDH2 genes but are more frequent in these tumours without IDH1/IDH2 mutations (Yan et al. 2009). Additionally, homozygous deletions of CDKN2A have been reported to define a subset of malignant astrocytomas in children (Schiffman et al. 2010). When deletion of CDKN2A is detected in combination with the BRAF p.V600E mutation in paediatric low grade glioma, there is an increased risk for transformation to secondary high grade glioma; therefore, therapy may need other preventive measures for this defined clinical distinct subgroup (Mistry et al. 2015).					
388	19508-L26462	MIR31	9p21.3	AAAGATGGCAAT-ATGTTGGCATAG	456.1 kb
285	16533-L26121	CDKN2A, ex 3 (4)	NM_000077.5; 33 nt before exon 3; NM_058195.4; 33 nt before exon 3	TTGACCTCAGGT-TTCTAACGCCTG	6.7 kb
249	10333-L17690	CDKN2A, upstream (intron 1)	NM_000077.5; 138 nt before exon 1; NM_058195.4; 3.8 kb before exon 2	GCCTGGAAAGAT-ACCGCGGTCCCT	25.6 kb
454	01531-L13742	CDKN2B	9p21.3	CCTAGGAAAGGT-GATAGAGCTTAG	-
IDH2 p.R172M and p.R172K, at 15q26.1 These probes will only give a signal when either the p.R172K or the p.R172M mutation is present in the sample. The p.R172K (c.515G>A) mutation has been detected by sequencing in 20 samples, and the p.R172M (c.515G>T) mutation in six samples, in a cohort of 1010 diffuse gliomas					

Length (nt)	MLPA probe	Gene (exon) ^a	Location (hg18) / Ligation site ^b	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
(Hartmann et al. 2009). The same study suggests that <i>IDH2</i> mutations occur predominantly in oligodendroglial tumours. No probe is present for the <i>IDH2</i> p.R172W (c.514A>T) mutation, which was detected in five samples in this study of 1010 patients.					
244 §	20963-L29001	<i>IDH2</i> , ex 4 (5) c.515G>T=p.R172M	NM_002168.4; 593-594	TACCATTGGCAT-GCACGCCCATGG	0.0 kb
238 §	20963-L29002	<i>IDH2</i> , ex 4 (5) c.515G>A=p.R172K	NM_002168.4; 593-594	TACCATTGGCAA-GCACGCCCATGG	-

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

^b Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.

^c Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

§ Mutation-specific probe. This probe will only generate a signal when the *BRAF* p.V600E, *IDH1* p.R132H / p.R132C or *IDH2* p.R172M / p.R172K mutation is present.

§ Please note that this probe might give a small signal on a sample with the *BRAF* p.V600K mutation.

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

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

– Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

SNVs located in the target sequence of a probe can influence probe hybridisation 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)	MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
346	05273-L04655	<i>SPAST</i>	2p22	CGAGCCACAGCA-AAAAGAGCCCTC	02-032.215
130	19551-L31871	<i>DYSF</i>	2p13	CCATTGCCAAGA-AGGTCAGTGTCC	02-071.750
417	13817-L15311	<i>EDAR</i>	2q13	TGGCCAGGTGAA-CCAGCGACAGCA	02-108.891
143	16316-L31755	<i>RAB7A</i>	3q21	CACAATAGGAGC-TGACTTTCTGAC	03-130.000
500	19675-L27812	<i>ATP8A1</i>	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042.278
124	18709-L21056	<i>IL4</i>	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132.038
463	10685-L31869	<i>PKHD1</i>	6p12	TCTGGCATCTAT-ATCTGCAGTCCC	06-051.876
280	13350-L26120	<i>PCSK5</i>	9q21	CATTAGCAAGCA-TTAGAACATCTC	09-077.989
310	16559-L26125	<i>SHANK2</i>	11q13	TGGTGCCAACAA-GGACTCACTCTC	11-070.331
382	09717-L31870	<i>NOS1</i>	12q24	GCTTGCAGATAT-GCATACAGCAGG	12-116.200
196	18049-L22439	<i>PLCG2</i>	16q23	TCCTGTCCGCCAG-CTGAGGAGGCGG	16-080.548
232	16428-L25931	<i>MYO5B</i>	18q21	TGGACCCTGATT-GATTTTATGAT	18-045.743
494	19137-L26747	<i>PSMG1</i>	21q22	TGGAAGCTTTTA-AGCCTATACTTT	21-039.471

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

Related products

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P370 product history	
Version	Modification
C1	One <i>RAF1</i> target probe and four reference probes have been replaced, and several probes have a change in length but no change in the sequence targeted. SALSA Binning DNA for this probemix has been changed from SD043 to SD054.
B1	Several new probes have been included for <i>FGFR1</i> , <i>MYB</i> and <i>MYBL1</i> , several probes have been replaced for 3p, 7q and 9p arms and most of the reference probes have been replaced. Also, the control fragments have been replaced (QDX2).
A1	First release.

Implemented changes in the product description
Version C1-06 – 8 September 2026 (05P) - Exon numbering section has been adjusted to update all gene transcripts to MANE transcripts. - Exon numbering of the <i>IDH1</i>, <i>SRGAP3</i>, <i>TACC1</i>, <i>IDH2</i> genes has changed. - Ligation sites of the probes targeting the <i>IDH1</i>, <i>SRGAP3</i>, <i>RAF1</i>, <i>FGFR1</i> genes updated according to the latest NM_ reference sequences. Version C1-05 – 04 November 2025 (05P) - Product description adapted to a new template. - Minor textual change in the Exon numbering section. - Description of the position of the 249 nt probe changed (no change in actual target site). - Positive control DNA samples section: information moved to product page on website. - Removed Related SALSA MLPA products section. Version C1-04 – 17 January 2023 (02P) - Exon numbering of the <i>CDKN2A</i> gene has been changed according to MANE in Table 2. See also the explanation on page 2. - Ligation sites of the <i>CDKN2A</i> probes 16533-L26121 and 10333-L17690 have been updated. Version C1-03 – 10 January 2023 (02P) - Information about possible small signal for BRAF V600E mutation probe on a sample with the V600K mutation added to the P370 specific notes section and Tables 1 and 2. - One new reference added for the P370 probemix on page 10. Version C1-02 – 25 March 2021 (02P) - SD054 details (plasmid DNA is used instead of synthetic DNA) are updated on page 3. - New reference added for the P370 probemix on page 10.

More information: www.mrcholland.com; www.mrcholland.eu

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