Product Description
SALSA® MLPA® Probemix P105-E1 Glioma

To be used with the MLPA General Protocol.

Version E1
As compared to version D3, two *TERT* mutation-specific and two flanking probes have been added, three references probes have been replaced and several probes have a change in length but no change in the sequence targeted. For complete product history see page 13.

Catalogue numbers
- **P105-025R:** SALSA® MLPA® Probemix P105 Glioma, 25 reactions
- **P105-050R:** SALSA® MLPA® Probemix P105 Glioma, 50 reactions
- **P105-100R:** SALSA® MLPA® Probemix P105 Glioma, 100 reactions

SALSA® MLPA® Probemix P105 Glioma (hereafter: P105 Glioma) 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.)

P105 Glioma can be used in combination with:

- SALSA® Binning DNA SD097 (Cat. No: SD097)

Volumes and ingredients

Volumes			Ingredients
P105-025R	P105-050R	P105-100R	
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	-25°C -15°C	☀

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 P105 Glioma is a **research use only (RUO)** assay for the detection of deletions or gains in the following genes *PDGFRA* (4q12), *EGFR* (7p11.2), *CDKN2A* (9p21.3), *PTEN* (10q23.31), *CDK4-MIR26A2-MDM2* (12q14-q15), *NFKBIA* (14q13.2) and *TP53* (17p13.1). Moreover, this probemix can be used to detect the chr. 7 gains and chr. 10 losses and to detect the presence of *TERT* promoter mutations C228T and C250T.

Gliomas are the most common primary brain tumours and account for one third of central nervous system (CNS) tumours. Gliomas comprise a very heterogeneous group of CNS neoplasms derived from glial cells. There are several oncogenes and tumour suppressor genes, which have been shown to undergo copy number changes in gliomas. Somatic mutations, disruptions, or copy number aberrations in three critical signalling pathways, a) the RTK/PI3K pathway (involving e.g. *EGFR, PDGFRA* and *PTEN* genes), b) the p53 pathway (involving e.g. *CDKN2A, MDM2* and *TP53* genes) and c) the RB pathway (involving e.g. *CDKN2A* and *CDK4* genes), are suggested to contribute to the development of gliomas (Cancer Genome Atlas Research Network 2008). Please see Table 2 for more details.

Epidermal growth factor receptor (EGFR) and its ligands are cell signalling molecules involved in diverse cellular functions. These include cell proliferation, differentiation, motility and survival, and tissue development. Glioblastomas often express EGFR variant III (EGFRvIII), a constitutively active genomic deletion variant of *EGFR* which is characterised by deletions of exons 2-7 of the *EGFR* gene (Sugawa et al. 1990). This probemix allows detection of deletions of *EGFR* that result in EGFRvIII. Please see Table 2 for more details.

Point mutations in *TERT* promoter region generate novel transcription factor binding sites and thus increase the expression of telomerase enzyme encoded by *TERT*. Common *TERT* promoter mutations are known as C228T and C250T, referring to C>T transitions at hg19/GCRh37 chr5:1295<u>228</u> and chr5:1295<u>250</u> positions, respectively. These mutations are predominantly present in oligodendroglioma and are associated with poor prognosis and reduced survival in the absence of *IDH*-mutation (Labussière et al. 2014). *TERT* promoter mutation, in combination with *IDH*-mutation and 1p/19q codeletion, is characteristic of oligodendroglioma. Absence of *TERT* promoter mutation, coupled with the presence of *IDH*-mutation, designates astrocytoma (Cahill et al. 2015; Eckel-Passow et al. 2015).

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
Tark – Transcript Archive: https://tark.ensembl.org

Exon numbering

The exon numbering used in this P105-E1 Glioma product description is the exon numbering derived from MANE project based on MANE Select transcripts for *PDGFRA*, *EGFR, PTEN*, *CDK4*, *MDM2*, *TP53 and NFKBIA* genes.. **From product description version D3-03 onwards, the exon numbering from the MANE transcripts is used for *CDKN2A*.** Consequently, for *CDKN2A*, the exon numbering is as follows: NM_000077.5 (MANE Select) encoding p16INK4A and NM_058195.4 (MANE Plus Clinical) encoding p14ARF are used. 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 exon numbering used in previous versions of this product description, can be found in between brackets in the Table 2. **Please be aware that the MANE and LRG exon numbering do not always correspond, and MANE exon numbering used here may differ from literature.** Please note that exon numbering for the same gene might be different in other MRC Holland product descriptions, where other resources used for exon numbering are indicated. 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 P105-E1 Glioma contains 60 MLPA probes with amplification products between 120 and 500 nucleotides (nt). This includes in total 43 probes for the *PDGFRA*, *EGFR*, *CDKN2A*, *PTEN*, *CDK4, MIR26A2, MDM2*, *NFKBIA* and *TP53* genes, and single flanking probes for 7q and 10p arms. Furthermore, this probemix contains two probes specific for the *TERT* C228T and C250T mutations, which will only generate a signal when the mutation is present. In addition, 13 reference probes are included that detect relatively copy number stable regions in various cancer types including gliomas. 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)	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 healthy individuals without a history of glioma should be included in each MLPA experiment for data normalisation. 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 P105 product page on our website.

SALSA® Binning DNA SD097

SALSA® Binning DNA SD097 provided with this probemix can be used for binning of all probes including the two mutation-specific probes (126 nt probe S1310-L32991 for *TERT* C250T and 156 nt probe 23341-L33133 for *TERT* C228T). SD097 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 µl SD097 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 SD097 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 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/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.

* Final ratios of >2.15 may be indicative of an amplification (gain of >2 copies).

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 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 or in or near the *CDK4, EGFR, NFKBIA* genes. 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.

P105 Glioma specific notes

- 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 CNAs, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.
- The *TERT* promoter mutation-specific probes are only intended to determine the presence (or absence) of the mutation.

Limitations of the procedure

- In many tumour samples, genetic alterations in *PDGFRA, EGFR, CDKN2A, PTEN, CDK4, MIR26A2, MDM2, NFKBIA* and *TP53* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P105 Glioma. The two most common promoter point mutations in the *TERT* gene can be detected, but other point mutations in the *TERT* gene 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 CNA in a patient sample, especially in solid tumours with more chaotic karyotypes.

Confirmation of results
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 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 *EGFR* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. P105-E1 Glioma

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]				Location (hg18) in kb
		Reference	*EGFR*	*PTEN*	Other targets	
64-105	Control fragments – see table in probemix content section for more information					
120 ¥	Reference probe 18946-L28961	5q31				05-132.038
126 * §	**TERT probe** S1310-L32991				**C250T mutation-specific**	05-001.348
133 ¥	Reference probe 16316-L20697	3q21				03-130.000
137	**EGFR probe** 06121-L20393		Exon 6			07-055.188
142	**CDKN2A probe** 18753-L24594				9p21.3	09-021.985
148 *	Reference probe 05795-L05242	15q15				15-040.479
153 ¥ «	**NFKBIA probe** 23396-L33115				14q13.2	14-034.942
156 * §	**TERT probe** 23341-L33133				**C228T mutation-specific**	05-001.348
161	**EGFR probe** 05438-L24607		Exon 5			07-055.186
167	**TP53 probe** 01588-L06028				17p13.1	17-007.531
172 «	**EGFR probe** 06405-L24605		Exon 1			07-055.054
178	**PTEN probe** 17314-L20922			Intron 3		10-089.675
185 ¥	**EGFR probe** 23288-L32941		Exon 7			07-055.189
190±	**PTEN probe** 06729-L06339			Intron 2		10-089.644
196	**TP53 probe** 01996-L09268				17p13.1	17-007.521
202	Reference probe 04542-L03931	2q24				02-166.567
208 ±	**PTEN probe** 17391-L21278			Exon 4		10-089.681
214 #	**PTEN probe** 07686-L15591			Exon 9		10-089.716
220	**EGFR probe** 17208-L23696		Exon 4			07-055.182
226	**PTEN probe** 17387-L24930			Exon 1		10-089.614
232 «	**NFKBIA probe** 18757-L24608				14q13.2	14-034.942
238 *	Reference probe 05709-L33003	3q21				03-123.484
244	Reference probe 12431-L13432	22q12				22-032.003
250	**CDKN2A probe** 16060-L22417				9p21.3	09-021.965
256	**EGFR probe** 05959-L24612		Exon 13			07-055.197
263 ‡	**TP53 probe** 02376-L21409				17p13.1	17-007.519
269	**EGFR probe** 05969-L24610		Exon 23			07-055.234
277 «	**NFKBIA probe** 18759-L24127				14q13.2	14-034.942
283 ‡	**TP53 probe** 01999-L21074				17p13.1	17-007.518
290 * ¬	Flanking probe 23340-L26861				10p11.21	10-034.666
294 ¥	Reference probe 11900-L23408	6p12				06-052.028
299	**TP53 probe** 17420-L29693				17p13.1	17-007.520
309	**CDKN2A probe** 17814-L22631				9p21.3	09-021.958
316	**TP53 probe** 17421-L24613				17p13.1	17-007.519
324	**PTEN probe** 03639-L24076			Exon 6		10-089.702
331	Reference probe 08905-L24614	11p11				11-047.316
340 ¥ «	**CDK4 probe** 17815-L32990				12q14.1	12-056.431
346	**EGFR probe** 17210-L24618		Exon 16			07-055.206
353	**TP53 probe** 17422-L24617				17p13.1	17-007.515
360 #	**PTEN probe** 17397-L24616			Exon 8		10-089.711
366 *	Reference probe 10086-L33004	8q22				08-100.723
373 #	**PTEN probe** 03638-L22839			Exon 5		10-089.683
380 * ¬	Flanking probe 23339-L28370				7q21.2	07-092.085
385	**CDKN2A probe** 17817-L23295				9p21.3	09-021.961
391	**PDGFRA probe** 03107-L02038				4q12	04-054.822
399	**EGFR probe** 05436-L24070		Exon 3			07-055.178
407	**PDGFRA probe** 18755-L24123				4q12	04-054.851
413	**MDM2 probe** 18255-L32191				12q15	12-067.517
421	**EGFR probe** 21280-L27637		Exon 2			07-055.178

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]				Location (hg18) in kb
		Reference	*EGFR*	*PTEN*	Other targets	
427	Reference probe 08839-L32428	2p13				02-071.767
436	**EGFR probe** 02063-L32279		Exon 8			07-055.191
443	**PDGFRA probe** 18756-L32278				4q12	04-054.826
450	**TP53 probe** 17424-L27139				17p13.1	17-007.514
454 «	**CDK4 probe** 18752-L32277				12q14.1	12-056.428
463	Reference probe 15970-L32276	18p11				18-012.784
472	**MIR26A2 probe** 18710-L32275				12q14.1	12-056.505
479 #	**PTEN probe** 17386-L32274			Exon 7		10-089.708
486	**MDM2 probe** 07178-L32273				12q15	12-067.489
493	Reference probe 16456-L24172	18q21				18-045.630
500	Reference probe 17001-L22947	20q11				20-034.954

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

* New in version E1.
¥ Changed in version E1. Minor alteration, no change in sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the *TERT* C250T or C228T, mutation is present. It has been tested on artificial and cell line DNA **but not on positive human samples!**
« 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.
± SNV rs146326040 could influence the probe signal at 190 nt, and SNV rs587780544 could influence the probe signal at 208 nt. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
‡ The ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by the IARC TP53 Database (https://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. CNAs 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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P105-E1 probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene / exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
PDGFRA **gene, at 4q12, indicated ligation sites are according to NM_006206.6.** *PDGFRA* amplification or gain is detected in 2-18% of gliomas (Alentorn et al. 2012; Bleeker et al. 2012; Cancer Genome Atlas Research Network, 2008). The frequency of *PDGFRA* amplification is shown to increase with tumour grade, and the amplification of *PDGFRA* is suggested to be a poor prognostic factor in anaplastic gliomas and in *IDH1*-mutant *de novo* glioblastoma multiforme (GBMs) (Alentorn et al. 2012; Phillips et al. 2013).					
391	03107-L02038	*PDGFRA*, ex 3	299-300	GGAGAGTGAAGT-GAGCTGGCAGTA	3,8 kb
443	18756-L32278	*PDGFRA*, ex 5	846-847	ACCTGTGCTGTT-TTTAACAATGAG	25,4 kb
407	18755-L24123	*PDGFRA*, ex 22	3067-3068	ATCCTGCTGTGG-CACGCATGCGTG	-
TERT, at 5p15.33. Ligation sites are according to NM_198253.3. The probes at 156 nt and 126 nt will only give a signal when respectively the C228T and C250T mutation is present in the sample. These mutations are also known with other names in literature and databases, with the most common ones being NM_198253.2: c.1-124C>T or C>T at chr5:1295228 (hg19, reverse strand) for C228T; and NM_198253.2: c.1-146C>T or C>T at chr5:1295250 (hg19, reverse strand) for C250T.					
126 §	S1310-L32991	*TERT*, ex 1	**C250T**; 67 nt before exon 1	CGGGGACCCGG**A**-AGGGGTCGGGAC	22 bp
156 §	23341-L33133	*TERT*, ex 1	**C228T**; 45 nt before exon 1	GAGGGCCCGGA**A**-GGGGCTGGGCGG	-

Length (nt)	MLPA probe	Gene / exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
EGFR gene, at 7p11.2, indicated ligation sites are according to NM_005228.5. *EGFR* amplification is frequently detected in primary glioblastomas and is associated with poor prognosis (Hurtt et al. 1992). Glioma patients with amplification of wt-*EFGR* have been suggested to respond well to EGFR kinase inhibitors, especially in combination therapy (Joshi et al. 2012). **EGFR variant III** (EGFRvIII) is an oncogenic, constitutively active mutant form of *EGFR* that is commonly expressed in glioblastoma. EGFRvIII is formed by an in-frame genomic deletion of exons 2 to 7 of *EGFR*, producing a truncated receptor lacking a portion of the extracellular ligand binding domain (Gan et al. 2013). The expression of EGFRvIII is suggested to confer worse prognosis than wt-*EGFR* expression alone (Shinojima et al. 2003). One flanking probe for *CDK6* gene is locating on 7q arm and allowing the detection of chromosome 7 copy number. *CDK6* gains and amplifications are detected glioblastoma and astrocytoma (Neil et al. 2020).					
172 «	06405-L24605	*EGFR*, ex 1	223-224	CCGTCCAGTATT-GATCGGGAGAGC	123,1 kb
421	21280-L27637	*EGFR*, ex 2	440-441	TAACTGTGAGGT-GGTCCTTGGGAA	1,0 kb
399	05436-L24070	*EGFR*, ex 3	533-534	TTATGTCCTCAT-TGCCCTCAACAC	3,4 kb
220	17208-L23696	*EGFR*, ex 4	817-818	ACCACCTGGGCA-GCTGTAAGTGTC	4,6 kb
161	05438-L24607	*EGFR*, ex 5	852-853	TGTCCCAATGGG-AGCTGCTGGGGT	1,2 kb
137	06121-L20393	*EGFR*, ex 6	914-915	CTGTGCCCAGCA-GTGCTCCGGGCG	1,5 kb
185	23288-L32941	*EGFR*, ex 7	1117-1118	AGGGCAAATACA-GCTTTGGTGCCA	1,8 kb
436	02063-L32279	*EGFR*, ex 8	1215-1216	AGCTATGAGATG-GAGGAAGACGGC	5,7 kb
256	05959-L24612	*EGFR*, ex 13	1862-1863	CCGAGGCAGGGA-ATGCGTGGACAA	9,6 kb
346	17210-L24618	*EGFR*, ex 16	2169-2170	CTTGAAGGCTGT-CCAACGAATGGG	27,6 kb
269	05969-L24610	*EGFR*, ex 23	3037-3038	AGATCTCCTCCA-TCCTGGAGAAAG	36,9 **Mb**
380 ¬	23339-L28370	*CDK6*	7**q**21.2	GAGAAGAAGACT-GGCCTAGAGATG	-
CDKN2A gene, at 9p21.3. Deletion or mutation of *CDKN2A* is detected in ~50% of glioma samples (Beroukhim et al. 2007; Cancer Genome Atlas Research Network, 2008) and a homozygous loss of *CDKN2A* is suggested to be a progression-associated genetic marker in glioma (Appay et al. 2019, Lu et al. 2020).					
309	17814-L22631	*CDKN2A*, ex 3 (4)	NM_000077.5; 830-831; NM_058195.4; 904-905	TTGCGAGCCTCG-CAGCCTCCGGAA	3,0 kb
385	17817-L23295	*CDKN2A*, ex 2 (3)	NM_000077.5; 430-431; NM_058195.4; 504-505	TGCGCGCGGCTG-CGGGGGGCACCA	4,1 kb
250	16060-L22417	*CDKN2A*, up (intron 1)	NM_000077.5; 138 nt before ex 1; NM_058195.4; 3.8 kb before ex 2	GCCTGGAAAGAT-ACCGCGGTCCCT	19,6 kb
142	18753-L24594	*CDKN2A*, up (1)	NM_000077.5; 19.7 kb before ex 1; NM_058195.4; 175 nt before ex 1	CGCAGGGCTCAG-AGCCGTTCCGAG	-
PTEN gene, at 10q23.31, indicated ligation sites are according to NM_000314.8. Monosomy or LOH of chr. 10 or 10q loss is the most common genomic alteration found in primary and secondary glioblastomas (Ohgaki et al. 2004; Beroukhim et al. 2007). Co-expression of *EGFRvIII* and *PTEN* has been suggested to associate with favourable clinical response to EGFR kinase inhibitors (Mellinghoff et al. 2005), while loss of *PTEN* expression seems to correlate with resistance to gefitinib (Guillamo et al. 2009). One flanking probe on 10p arm aids in the detection of chromosome 10 copy number.					
290 ¬	23340-L26861	*PARD3*	10**p**11.21	TGGATTTCGTTA-AAACACGAAAAT	54,9 **Mb**
226	17387-L24930	*PTEN*, ex 1	781-782	CCTGCAGAAGAA-GCCCCGCCACCA	29,9 kb
190 ±	06729-L06339	*PTEN*, intr. 2 (ex 2)	217 nt after ex 2 reverse	TATCACATAAGT-ACCTGATTATGT	31,5 kb
178	17314-L20922	*PTEN*, intr. 3 (ex 3)	226 nt after ex 3	TTGATCTGCTTT-AAATGACTTGGC	5,2 kb
208 ±	17391-L21278	*PTEN*, ex 4	14 nt before ex 4 reverse	AAAAGAAAAGTT-TAAAAGTGATAT	2,1 kb
373 #	03638-L22839	*PTEN*, ex 5	1250-1251	GGTGTAATGATA-TGTGCATATTTA	19,1 kb
324	03639-L24076	*PTEN*, ex 6	1473-1472 reverse	CTTACTGCAAGT-TCCGCCACTGAA	5,7 kb
479 #	17386-L32274	*PTEN*, ex 7	1550-1551	ACACGACGGGAA-GACAAGTTCATG	3,1 kb
360 #	17397-L24616	*PTEN*, ex 8	1787-1788	AATGACAAGGAA-TATCTAGTACTT	5,4 kb
214 #	07686-L15591	*PTEN*, ex 9	3003-3002 reverse	ACAGCATCTGAA-TTTTAGCACTGG	-
CDK4, ***MIR26A2*** and ***MDM2***, at 12q14.1-q15 Amplification of 12q14-q15, which harbours the *CDK4* and *MDM2* genes, is detected in 14-18% of newly diagnosed glioblastoma patients (Cancer Genome Atlas Research Network, 2008). The *MIR26A2* gene (at 12q14.1) is also shown to be amplified in high-grade glioma, and this amplification is correlated with monoallelic *PTEN* deletion (Huse et al. 2009). In addition, *MIR26A2* is shown to regulate *PTEN* expression and, thereby, *MIR26A2* amplification provides a significant growth advantage for tumour cells (Kim et al. 2010).					

Length (nt)	MLPA probe	Gene / exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
454 «	18752-L32277	*CDK4*, ex 8	NM_000075.4; 1157-1158	TCTCTGAGGCTA-TGGAGGGTCCTC	2,5 kb
340 «	17815-L32990	*CDK4*, ex 4	NM_000075.4; 644-645	GGCCTGGCCAGA-ATCTACAGCTAC	73,7 kb
472	18710-L32275	*MIR26A2*	12q14.1	AGGCCTCACAGA-TGGAAACAGCCT	11,0 **M**b
486	07178-L32273	*MDM2*, ex 2	NM_002392.6; 355-356	CCTACTGATGGT-GCTGTAACCACC	27,5 kb
413	18255-L32191	*MDM2*, ex 10	NM_002392.6; 1195-1194 reverse	TCAGGATCTTCT-TCAAATGAATCT	-
NFKBIA gene, at 14q13.2, indicated ligation sites are according to NM_020529.3. Deletion of NF-κB inhibitor (*NFKBIA*) is detected in about 25% of glioblastomas (Bredel et al. 2011). It is suggested that deletion of *NFKBIA* and amplification of *EGFR* show a pattern of mutual exclusivity and that *NFKBIA* deletion could substitute for *EGFR* amplification (Bredel et al. 2011).					
232 «	18757-L24608	*NFKBIA*, ex 5	808-807 reverse	CTTCAACAGGAG-TGACACCAGGTC	0,2 kb
277 «	18759-L24127	*NFKBIA*, ex 4	706-707	GCATCGTGGAGC-TTTTGGTGTCCT	0,6 kb
153 «	23396-L33115	*NFKBIA*, ex 3	445-446	ATTCGTAGACTC-CACTCCACTTGG	-
TP53 gene, at 17p13.1, indicated ligation sites are according to NM_000546.6. Mutations or homozygous deletions of *TP53* are detected in 35% of newly diagnosed diffuse glioblastomas (Cancer Genome Atlas Research Network, 2008). Clinical and prognostic significance of *TP53* deletions is still under evaluation.					
450	17424-L27139	*TP53*, ex 11	1300-1301	CTCATGTTCAAG-ACAGAAGGGCCT	1,0 kb
353	17422-L24617	*TP53*, ex 10	1188-1189	TGAGGCCTTGGA-ACTCAAGGATGC	3,1 kb
283 ‡	01999-L21074	*TP53*, ex 8 (7)	981-982	CTGTCCTGGGAG-AGACCGGCGCAC	1,2 kb
316	17421-L24613	*TP53*, ex 6(5)	735-736	TATCCGAGTGGA-AGGAAATTTGCG	0,3 kb
263 ‡	02376-L21409	*TP53*, ex 5 (4b)	546-547	CAAGATGTTTTG-CCAACTGGCCAA	0,8 kb
299	17420-L29693	*TP53*, ex 4 (3)	451-450 reverse	TAGCTGCCCTGG-TAGGTTTTCTGG	0,6 kb
196	01996-L09268	*TP53*, ex 2 (2a)	117-118	CTCTTGCAGCAG-CCAGACTGCCTT	10,8 kb
167	01588-L06028	*TP53*, ex 1	58-59	TCCGGGGACACT-TTGCGTTCGGGC	-

[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.

§ Mutation-specific probe. This probe will only generate a signal when the *TERT* C250T or C228T, mutation is present. It has been tested on artificial and cell line DNA **but not on positive human samples!**

« 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.

± SNV rs146326040 could influence the probe signal at 190 nt, and SNV rs587780544 could influence the probe signal at 208 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

‡ The ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by the IARC TP53 Database (https://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. CNAs 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.

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)	MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
427	08839-L32428	*DYSF*	2p13	TGCCATGAAGCT-GGTGAAGCCCTT	02-071.767
202	04542-L03931	*SCN1A*	2q24	AACACCACAACT-GGTGACAGGTTT	02-166.567
238	05709-L33003	*CASR*	3q21	AGTGTGTGGAGT-GTCCTGATGGGG	03-123.484
133	16316-L20697	*RAB7A*	3q21	CACAATAGGAGC-TGACTTTCTGAC	03-130.000
120	18946-L28961	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132.038
294	11900-L23408	*PKHD1*	6p12	TGCTCTCTGGAT-TCAAGACTGAAA	06-052.028
366	10086-L33004	*VPS13B*	8q22	GCCAGTCAGCAT-CGCATTGCCCGT	08-100.723

Length (nt)	MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
331	08905-L24614	*MYBPC3*	11p11	CGTGGGAGAGGA-CTCCTGCACAGT	11-047.316
148	05795-L05242	*CAPN3*	15q15	TGGAGATCTGCA-ACCTCACGGCCG	15-040.479
463	15970-L32276	*PTPN2*	18p11	TCTGCACTAGTA-ACTGACAGTGCT	18-012.784
493	16456-L24172	*MYO5B*	18q21	TCTGACTCATCA-TCTCCCACTTCC	18-045.630
500	17001-L22947	*SAMHD1*	20q11	CCCTGTCACCTC-AAGTTTGAGGAT	20-034.954
244	12431-L13432	*LARGE1*	22q12	ATCCACCTGGTA-TGGTCGACGGGG	22-032.003

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

Related products
For related products, see the product page on our website.

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P105 product history	
Version	*Modification*
E1	Two *TERT* mutation specific probes added, flanking probes added to 7q and 10p arms, several reference probes replaced and several probes have a change in length but no change in the sequence targeted.
D3	One reference probe has been replaced and one reference probe has been added, and several probes have a change in length but no change in the sequence targeted.
D2	Two probes have a small change in length, but no change in the sequence targeted.
D1	Several target probes have been replaced, probes for new target genes/regions have been added (*PDGFRA*, *NFKBIA*, 12q14-q15), and most of the reference probes have been replaced.
C2	The 88 and 96 nt control fragments have been replaced (QDX2).
C1	One *EGFR* probe is replaced; four extra reference probes have been included, four probes have a slightly different length and two extra control fragments at 100-105 have been added.
B1	One *PTEN* probe has been replaced by two new probes.
A1	First release.

Implemented changes in the product description

Version E1-02 — 15 May 2025 (05P)

- Description of the position of the 178 nt, 190 nt and 250 nt probe changed (no change in actual target sites).
- Positive control DNA samples section: information moved to product page on website.
- Exon numbering of the TP53 gene has been changed according to MANE in Table 2.

Version E1-01 -30 September 2024 (05P)

- Product description adapted to a new product version (version number changed, changes in Table 1, Table 2 and Table 3) and to a new template.
- Probemix name has changed to “Glioma” (was “Glioma-2”).
- New cancer cell lines added to Positive control DNA samples table on page 4.
- P105 Glioma specific notes for *TERT* mutation detection added on page 6.
- SNV warning adjusted under the Table 1 and 2.

- New references added on pages 13-14.

Version D3-03 – 17 January 2023 (04P)
- Exon numbering of the *CDKN2A* gene has been changed according to MANE in Table 1 and 2. See also explanation on page 2.
- Ligation site of the CDKN2A probe 18753-L24594 has been updated.

Version D3-02 – 04 May 2021 (04P)
- Remark added, in section Positive control DNA samples, that some reference probes are affected by CNAs in the indicated cell line samples.

Version D3-01 – 21 April 2021 (04P)
- Product description adapted to a new product version (version number changed, changes in Table 1, Table 2 and Table 3) and adapted to a new template.
- Positive samples added on page 3.
- Ligation sites of the probes targeting the *PDGFRA, CDKN2A, PTEN, CDK4, MDM2, NFKBIA* and *TP53* genes updated according to newest version of the NM_ reference sequence.
- Warning added to Table 1 and 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- New references added on pages 10-11.
- Various minor textual and layout changes.

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