Product Description

SALSA® MLPA® Probemix P175-B2 Tumour Gain

To be used with the MLPA General Protocol.

Version B2
As compared to version B1, one reference probe has been replaced. For complete product history see page 12.

Catalogue numbers

- **P175-025R:** SALSA® MLPA® Probemix P175 Tumour Gain, 25 reactions
- **P175-050R:** SALSA® MLPA® Probemix P175 Tumour Gain, 50 reactions
- **P175-100R:** SALSA® MLPA® Probemix P175 Tumour Gain, 100 reactions

SALSA® MLPA® Probemix P175 Tumour Gain (hereafter: P175 Tumour Gain) 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™

P175 Tumour Gain can be used in combination with:

- SALSA® Binning DNA SD029 (Cat. No: SD029)

Volumes and ingredients

Volumes			Ingredients
P175-025R	**P175-050R**	**P175-100R**	
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		

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 P175 Tumour Gain is a **research use only (RUO)** assay for the detection of copy number aberrations in 24 genes, which are frequently gained or amplified in various tumour types. This probemix can also be used to detect the presence of the *BRAF* p.V600E (c.1799T>A) point mutation.

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 P175-B2 Tumour Gain product description for all genes is the exon numbering derived from MANE project (release version 1.4) based on MANE Select transcripts as indicated in Table 2. The *ABL1*, *ERBB2,* and *AURKA* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version B2-01 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select NM_005157.6 for *ABL1,* NM_004448.4 for *ERBB2*, and NM_198437.3 for *AURKA.* 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 in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
P175-B2 Tumour Gain contains 62 MLPA probes with amplification products between 115 and 504 nucleotides (nt). This includes two probes for each of the following genes: *ABL1*, *ALK*, *AR, AURKA/B*, *BRAF*, *CCND1/2*, *CDK4*, *DHFR*, *EGFR*, *ERBB2*, *FGFR1*, *KDR*, *KIT*, *MDM2/4*, *MET*, *MYC*, *MYCN*, *PDGFRA*, *RET*, *SMO* and *TOP2A*. Furthermore, this probemix also contains one probe specific for the *BRAF* p.V600E (c.1799T>A) point mutation which will only generate a signal when the mutation is present. In addition, 13 reference probes are included that relatively copy number stable regions in various cancer types. Partial probe sequences are available in Table 2 and 3, and online (www.mrcholland.com). The identity of the genes detected by the reference probes is available 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 individuals without a history of cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Samples from the Coriell Institute and Leibniz Institute DSMZ listed in the table below have been tested at MRC Holland with the P175-B2 probemix and can be used to detect copy number alterations (CNAs) in the genes targeted by this probemix. The mutation-specific probe can only detect the presence of the mutation and should not be used to determine zygosity. The quality of cell lines can change; therefore deviations to CNA findings might occur.

Sample name	Chromosomal position (hg18) of CNA*	Altered target genes in P175-B2 Tumour Gain	Expected CNA / Point mutation
Germline samples from Coriell Institute.			
NA05347	1q32.1	*MDM4*	Heterozygous duplication
NA10401[†]	2p23.2-p24.3	*MYCN, ALK*	Heterozygous duplication
NA00945	2p24.3	*MYCN*	Heterozygous deletion
NA07081	7p11.2	*EGFR*	Heterozygous duplication
NA01059	7q31.2	*MET*	Heterozygous deletion
NA12519	7q31.2-q34	*MET, SMO, BRAF*	Homozygous duplication/ Heterozygous triplication
NA07412	7q34	*BRAF*	Heterozygous deletion
NA02030	8p11.23-q24.21	*FGFR1, MYC*	Heterozygous duplication
NA03999	8q24.21	*MYC*	Heterozygous deletion
NA13685	9q34.12	*ABL1*	Heterozygous duplication
NA07981	12p13.32	*CCND2*	Homozygous duplication / Heterozygous triplication
Cancer cell line samples from Leibniz Institute DSMZ.			
DU-4475 (ACC-427)[†#]	1q32.1	*MDM4*	gain
	7q34	*BRAF*	p.V600E (c.1799T>A)

Sample name	Chromosomal position (hg18) of CNA*	Altered target genes in P175-B2 Tumour Gain	Expected CNA / Point mutation
SU-DHL-8 (ACC-573)†	7p11.2-q34	*EGFR, MET, SMO, BRAF*	gain
	12p13.32-q14.1	*CCND2, CDK4*	
	20q13.2	*AURKA*	

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by P175-B2 Tumour Gain.
† Some of the reference probes are also affected by CNAs.
# In this sample ambiguous ratios between were found for all probes targeting 7q31.2-q34 (*MET*, *SMO*, *BRAF*), thus indicating a potential subclonal gain.

SALSA® Binning DNA SD029
The SALSA® Binning DNA SD029 provided with this probemix can be used for binning of all probes including one mutation-specific probe (*BRAF* p.V600E (c.1799T>A) probe 08780-SP0039-L08904). SD029 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 probe. Inclusion of one reaction with 5 µl SD029 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 signal. For further details, please consult the SD029 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 these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication/gain		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 the GC-rich regions. 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 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.

P175 Tumour Gain 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 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.
- The BRAF p.V600E (c.1799T>A) mutation-specific probe is only intended to determine the presence (or absence) of the mutation. However, due to high nucleotide sequence similarity of mutated p.V600E (GTG to GAG single nucleotide variation) and p.V600K (GTG to AAG double nucleotide variation) codons, the BRAF p.V600E probe included in this probemix might give a small signal on a sample with p.V600K (c.1798_1799delinsAA) mutation.

Limitations of the procedure

- In tumour samples, genetic alterations in many cancer genes are small (point) mutations, none of which will be detected by using P175 Tumour Gain. One common point mutation in the *BRAF* gene can be detected, but other point mutations in this or other 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 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 mutation database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://varnomen.hgvs.org.

Table 1. P175-B2 Tumour Gain

Length (nt)	MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
64-105	Control fragments – see table in probemix content section for more information			
115 *	Reference probe S0973-L26704	4p13		04-042.278
121	**DHFR probe** S0428-L27347		5q14.1	05-079.986
124	**AURKA probe** S0429-L27348		20q13.2	20-054.379
131	**AR probe** 21771-L13680		Xq12	X-066.823
136	Reference probe 13867-L30857	16p13		16-008.765
143 «	**CDK4 probe** 03173-L30917		12q14.1	12-056.431
148	**ERBB2 probe** 21772-L30858		17q12	17-035.122
152	Reference probe 14199-L25033	2q13		02-108.894
157	**MYC probe** 20780-L30918		8q24.21	08-128.822
161	**MET probe** 20064-L27635		7q31.2	07-116.187
167	**ABL1 probe** 12502-L30479		9q34.12	09-132.579
172	**ALK probe** 08324-L30480		2p23.2	02-029.405
176	**CCND2 probe** 03177-L30859		12p13.32	12-004.253
182	**RET probe** 21776-L30860		10q11.21	10-042.942
187	**MDM4 probe** 03185-L30861		1q32.1	01-202.761
191	**AURKB probe** 12749-L30862		17p13.1	17-008.051
196	Reference probe 05703-L29853	3q21		03-123.456
202	**MET probe** 10314-L30481		7q31.2	07-116.167
208	**SMO probe** 12750-L30482		7q32.1	07-128.633
214 #	**BRAF probe** 04260-L14063		7q34	07-140.123
220	Reference probe 06714-L30959	15q24		15-070.433
226 § Ж	**BRAF probe** 08780-SP0039-L08904		p.V600E (c.1799T>A)	07-140.100
232	**EGFR probe** 06408-L31001		7p11.2	07-055.217
238	**MYC probe** 21646-L19746		8q24.21	08-128.822
244	**DHFR probe** 12753-L13869		5q14.1	05-079.986
251	**BRAF probe** 10507-L11060		7q34	07-140.099
257	**TOP2A probe** 01055-L00628		17q21.2	17-035.823
265 «	**CDK4 probe** 15904-L30865		12q14.1	12-056.429
273	**CCND1 probe** 05401-L30866		11q13.2	11-069.167
282	Reference probe 13392-L30484	6q12		06-065.358
292	**MDM2 probe** 07179-L30485		12q15	12-067.494
299	**CCND1 probe** 00583-L30869		11q13.2	11-069.175
305	**KDR probe** 12755-L30870		4q12	04-055.657
312	**ABL1 probe** 12516-L30871		9q34.12	09-132.749
319	Reference probe 06580-L30872	2q24		02-165.907
325	**AR probe** 12604-L30873		Xq12	X-066.860
330	**MDM4 probe** 03186-L30874		1q32.1	01-202.779
337	Reference probe 20864-L28882	14q24		14-072.684
344	**ERBB2 probe** 00717-L30875		17q12	17-035.137
351	**KIT probe** 21774-L30876		4q12	04-055.257
357	**FGFR1 probe** 04439-L30877		8p12	08-038.393
363	Reference probe 14835-L29122	1p34		01-045.252
370	**RET probe** 18546-L30919		10q11.21	10-042.928
376 «	**MYCN probe** 02572-L30879		2p24.3	02-016.003
385	**FGFR1 probe** 01046-L24278		8p12	08-038.434
391	**PDGFRA probe** 12762-L13878		4q12	04-054.851
399	**CCND2 probe** 03178-L30880		12p13.32	12-004.283
406	**SMO probe** 12757-L30881		7q32.1	07-128.640
412	**MDM2 probe** 07180-L30490		12q15	12-067.497
418	Reference probe 20960-L30882	6p12		06-052.049
426	**ALK probe** 08323-L30883		2p23.2	02-029.608

Length (nt)	MLPA probe	Chromosomal position (hg18)		Location (hg18) in kb
		Reference	Target region	
430	**EGFR probe** 02063-L30920		7p11.2	07-055.191
438	**PDGFRA probe** 18756-L24124		4q12	04-054.826
445 «	**MYCN probe** 03327-L20117		2p24.3	02-016.003
454	**KDR probe** 12758-L31062		4q12	04-055.663
462	**AURKB probe** 12759-L30885		17p13.1	17-008.052
469	Reference probe 19978-L30964	4p16		04-005.637
475	**KIT probe** 12761-L30887		4q12	04-055.298
481	**TOP2A probe** 01056-L30888		17q21.2	17-035.801
489	**AURKA probe** 10236-L14068		20q13.2	20-054.390
496	Reference probe 17940-L30958	19p13		19-013.255
504	Reference probe 21229-L30802	10p11		10-032.800

* New in version B2.
§ Mutation-specific probe. This probe will only generate a signal when the *BRAF* p.V600E (c.1799T>A) mutation is present. It has been tested on artificial DNA and on a cell line DU-4475 (ACC-427), as shown in the 'Positive control DNA samples' section, **but not on positive human samples!** 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.
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. P175 probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene	Exon[a] / mutation	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
MDM4 gene at **1q32.1**; 11 exons, based on MANE select transcript NM_002393.5. No other MDM4 probes are present in our collection at this moment.					
187	03185-L30861	***MDM4***	Exon 2	TTCACTACCAAA-ATGACATCATTT	17,3 kb
330	03186-L30874	***MDM4***	Exon 8	GGAGTGGGATGT-AGCTGGCCTGCC	-
MYCN gene at **2p24.3**; 3 exons, based on MANE select transcript NM_005378.6. More MYCN probes are present in the P037 CLL-1 and P377 Hematologic Malignancies probemixes.					
376 «	02572-L30879	***MYCN***	Exon 3	CTGTCACCACAT-TCACCATCACTG	0,2 kb
445 «	03327-L20117	***MYCN***	Exon 3	TGCACCCCCACA-GAAGAAGATAAA	13,4 **Mb** to *ALK*
ALK gene at **2p23.2**; 29 exons, based on MANE select transcript NM_004304.5. More ALK probes are present in the P037 CLL-1 and P252 NB mix 2 probemixes.					
172	08324-L30480	***ALK***	Exon 6	TCACTTGTTGGA-ATGGGACAGTCC	203,7 kb
426	08323-L30883	***ALK***	Exon 4	ACACCTCAGCTG-ACTCCAAGCACA	79,3 **Mb** to ref probe
PDGFRA gene at **4q12**; 23 exons, based on MANE select transcript NM_006206.6. More PDGFRA probes are present in the P105 Glioma probemix.					
438	18756-L24124	***PDGFRA***	Exon 5	ACCTGTGCTGTT-TTTAACAATGAG	25,4 kb
391	12762-L13878	***PDGFRA***	Exon 22	ACAATGCATACA-TTGGTGTCACCT	405,3 kb to *KIT*
KIT gene at **4q12**; 21 exons, based on MANE select transcript NM_000222.3. No other KIT copy number probes are present in our collection at this moment. A mutation-specific probe for *KIT* p.D816V is present in P420 MPN mix 1 and P520 MPN mix 2 probemixes.					

Length (nt)	MLPA probe	Gene	Exon[a] / mutation	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
351	21774-L30876	***KIT***	Exon 2	CGTGCACCAACA-AACACGGCTTAA	41,5 kb
475	12761-L30887	***KIT***	Exon 20	ACATAATGAAGA-CTTGCTGGGATG	359,1 kb to *KDR*
KDR gene at **4q12**; 30 exons, based on MANE select transcript NM_002253.4. No other KDR probes are present in our collection at this moment.					
305	12755-L30870	***KDR***	Exon 19	TGGTGACCAATA-TGAATGAGGATC	6,2 kb
454	12758-L31062	***KDR***	Exon 14	GAAACCTGGAGA-ATCAGACGACAA	-
DHFR gene at **5q14.1**; 6 exons, based on MANE select transcript NM_000791.4. No other DHFR probes are present in our collection at this moment.					
121	S0428-L27347	***DHFR***	Exon 2	CGCTGTTTCTCT-AACTTGTAGGAA	0,8 kb
244	12753-L13869	***DHFR***	Exon 1	GGCTTCCCGTAG-ACTGGAAGAATC	-
EGFR gene at **7p11.2;** 28 exons, based on MANE select transcript NM_005228.5. More EGFR probes are present in the P078 Breast tumour, P105 Glioma, P315 EGFR, P477 Head and neck carcinoma, and P483 HER gene family probemixes.					
430	02063-L30920	***EGFR***	Exon 8	AGCTATGAGATG-GAGGAAGACGGC	25,5 kb
232	06408-L31001	***EGFR***	Exon 20	CCTCCTGGACTA-TGTCCGGGAACA	61,0 **Mb** to *MET*
MET gene at **7q31.2**; 21 exons, based on MANE select transcript NM_000245.4. More MET probes are present in the P308 MET probemix.					
202	10314-L30481	***MET***	Exon 4	TATCACTGGGAA-GAAGGTAAGCTG	19,3 kb
161	20064-L27635	***MET***	Exon 10	AGCACAATAACA-GGTGTTGGGAAA	12,4 **Mb** to *SMO*
SMO gene at **7q32.1**; 12 exons, based on MANE select transcript NM_005631.5. No other SMO probes are present in our collection at this moment.					
208	12750-L30482	***SMO***	Exon 4	CCCTGCTGTTAT-TCTCTTCTACGT	6,8 kb
406	12757-L30881	***SMO***	Exon 12	TCGGTGAGGAAG-AAGAGCCTTGAA	11,5 **Mb** to *BRAF*
BRAF gene at **7q34**; 18 exons, based on MANE select transcript NM_004333.6. More BRAF copy number probes are present in the P298 BRAF-HRAS-KRAS-NRAS and P370 BRAF-IDH1-IDH2 probemixes. A mutation-specific probe for *BRAF* p.V600E is present in ME011 Mismatch Repair Genes, ME042 CIMP, P298 BRAF-HRAS-KRAS-NRAS, P370 BRAF-IDH1-IDH2, and D006 Multiple Myeloma probemixes.					
251	10507-L11060	***BRAF***	Exon 15	TATTTTTCCACT-GATTAAATTTTT	0,1 kb
226 § Ж	08780-SP0039-L08904	***BRAF***	Exon 15 p.V600E (c.1799T>A)	TTCTTCATGAAG-ACCTCACAGTAAAA ATAGGTGATTTTGGTCTAGCTACAG**A**-GAAATCTCGATG	23,6 kb
214 #	04260-L14063	***BRAF***	Exon 13	CTTGTATCACCA-TCTCCATATCAT	-
FGFR1 gene at **8p12**; 18 exons, based on MANE select transcript NM_023110.3. More FGFR1 probes are present in the P370 BRAF-IDH1-IDH2, P080-C2 Craniofacial, P078 Breast tumour, and P133 Kallmann-2 probemixes.					
357	04439-L30877	***FGFR1***	Exon 13	ACCCCAGCCACA-ACCCAGAGGAGC	41,5 kb
385	01046-L24278	***FGFR1***	Exon 2	CAACCTCTAACT-GCAGAACTGGGA	90,4 **Mb** to *MYC*
MYC gene at **8q24.21**; 3 exons, based on MANE select transcript NM_002467.6. More MYC probes are present in the P037 CLL-1, P078 Breast tumour, P377 Hematologic Malignancies, and D006 Multiple Myeloma probemixes.					
238	21646-L19746	***MYC***	Exon 3	AGGACTATCCTG-CTGCCAAGAGGG	0,2 kb
157	20780-L30918	***MYC***	Exon 3	GAACGAGCTAAA-ACGGAGCTTTTT	-
ABL1 gene at **9q34.12**; 11 exons, based on MANE select transcript NM_005157.6. More ABL1 probes are present in the P383 T-ALL and D007 Acute Lymphoblastic Leukemia probemixes.					
167	12502-L30479	***ABL1***	Upstream (Exon 1)	CTTTATGTGTGA-GAATTGAAATGA	170,1 kb
312	12516-L30871	***ABL1***	Exon 11	TCGAAAAGAGCG-AGGTCCCCCGGA	-
RET gene at **10q11.21**; 20 exons, based on MANE select transcript NM_020975.6. More RET probes are present in the P169 Hirschsprung-1 probemix.					
370	18546-L30919	***RET***	Exon 8	TGCAGTCAGCAA-GAGACGGCTGGA	14,5 kb
182	21776-L30860	***RET***	Exon 19	CCTCCCTTCCAC-ATGGATTGAAAA	-

Length (nt)	MLPA probe	Gene	Exon[a] / mutation	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
CCND1 gene at **11q13.2**; 5 exons, based on MANE select transcript NM_053056.3. More CCND1 probes are present in both P078 Breast tumour, P477 Head and Neck Carcinoma, and D006 Multiple Myeloma probemixes.					
273	05401-L30866	***CCND1***	Exon 2	TCGCTGGAGCCC-GTGAAAAAGAGC	8,1 kb
299	00583-L30869	***CCND1***	Exon 5	CCCTGCTGGAGT-CAAGCCTGCGCC	-
CCND2 gene at **12p13.32**; 5 exons, based on MANE select transcript NM_001759.4. More CCND2 probes are present in the P037 CLL-1, P040 CLL, and P377 Hematologic Malignancies probemixes.					
176	03177-L30859	***CCND2***	Exon 1	AGACCAGTTTTA-AGGGGAGGACCG	29,9 kb
399	03178-L30880	***CCND2***	Exon 5	TAACAGCCAAGA-AGCCTGCAGGAG	52,2 **Mb** to *CDK4*
CDK4 gene at **12q14.1**; 8 exons, based on MANE select transcript NM_000075.4. More CDK4 probes are present in the P419 CDKN2A/2B-CDK4 probemix.					
265 «	15904-L30865	***CDK4***	Exon 8	TGCTGACTTTTA-ACCCACACAAGC	2,7 kb
143 «	03173-L30917	***CDK4***	Exon 3	AACCCTGGTGTT-TGAGCATGTAGA	11,1 **Mb** to *MDM2*
MDM2 gene at **12q15**; 11 exons, based on MANE select transcript NM_002392.6. More MDM2 probes are present in the P323 CDK4-HMGA2-MDM2 probemix.					
292	07179-L30485	***MDM2***	Exon 3	ACCAACAGACTT-TAATAACTTCAA	3,4 kb
412	07180-L30490	***MDM2***	Exon 4	TGGACTAAACTG-AAGAATTACCTG	-
AURKB gene at **17p13.1**; 9 exons, based on MANE select transcript NM_004217.4. No other AURKB probes are present in our collection at this moment.					
191	12749-L30862	***AURKB***	Exon 5	CCTTCCTCCACT-TTCTAAGCAGGC	0,2 kb
462	12759-L30885	***AURKB***	Exon 4	GCACTTACGTTA-AGATGTCGGGTG	27,1 **Mb** to *ERBB2*
ERBB2 gene, also known as *HER-2/NEU*, at **17q12**; 27 exons, based on MANE select transcript NM_004448.4. More ERBB2 probes are present in the P078 Breast tumour and P483 HER gene family probemixes.					
148	21772-L30858	***ERBB2***	Exon 8 (13)	AGGTGACAGCAG-AGGATGGAACAC	14,9 kb
344	00717-L30875	***ERBB2***	Exon 25 (30)	TCACTGCTGGAG-GACGATGACATG	664,7 kb to *TOP2A*
TOP2A gene at **17q21.2**; 35 exons, based on MANE select transcript NM_001067.4. More TOP2A probes are present in the P078 Breast tumour probemix.					
481	01056-L30888	***TOP2A***	Exon 33	TAAGGGCAGTGT-ACCACTGTCTTC	21,3 kb
257	01055-L00628	***TOP2A***	Exon 7	AAGCCCTTCAAT-GGAGAAGATTAT	-
AURKA gene at **20q13.2**; 9 exons, based on MANE select transcript NM_198437.3. More AURKA probes are present in the P078 Breast tumour probemix.					
124	S0429-L27348	***AURKA***	Exon 8 (10)	TACAAAAGAATA-TCACGGGTAAGA	11,1 kb
489	10236-L14068	***AURKA***	Exon 6 (8)	AGGCATCCTAAT-ATTCTTAGACTG	-
AR gene at **Xq12**; 8 exons, based on MANE select transcript NM_000044.6. More AR probes are present in the P074 AR probemix.					
131	21771-L13680	***AR***	Exon 3	AGCAGGGATGAC-TCTGGGAGGTAA	37,6 kb
325	12604-L30873	***AR***	Exon 8	CATCAGTTCACT-TTTGACCTGCTA	-

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

§ Mutation-specific probe. This probe will only generate a signal when the *BRAF* p.V600E (c.1799T>A) mutation is present. It has been tested on artificial DNA and on a cell line DU-4475 (ACC-427), as shown in the 'Positive control DNA samples' section, **but not on positive human samples!** 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.
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 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
363	14835-L29122	*UROD*	1p34	AAGCACCATGGC-TCAGGCCAAGCG	01-045.252
152	14199-L25033	*EDAR*	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108.894
319	06580-L30872	*SCN2A*	2q24	AACTTGGTTTGG-CAAATGTGGAAG	02-165.907
196	05703-L29853	*CASR*	3q21	GTGGCTTCCAAA-GACTCAAGGACC	03-123.456
469	19978-L30964	*EVC2*	4p16	AGACTCTGTCGG-CCTACACCGCCC	04-005.637
115	S0973-L26704	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042.278
418	20960-L30882	*PKHD1*	6p12	TTTATCCACCAA-GTGGTGTTCCAG	06-052.049
282	13392-L30484	*EYS*	6q12	AGCCAGCTGGTA-TGCACTAATGGG	06-065.358
504	21229-L30802	*CCDC7*	10p11	ATCGCCTTAAAC-AGAGGTCTAAAT	10-032.800
337	20864-L28882	*PSEN1*	14q24	TTTCTGTGAAAC-AGTATTTCTATA	14-072.684
220	06714-L30959	*HEXA*	15q24	TAGCCAGCTTGT-TTGGAAATCTGC	15-070.433
136	13867-L30857	*ABAT*	16p13	ACTTTGTGGAGA-AGCTCCGGCAGT	16-008.765
496	17940-L30958	*CACNA1A*	19p13	GCCATTACATCC-TGAACCTGCGCT	19-013.255

Related products

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

References

- 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.
- 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.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using P175 Tumour Gain

- Barbieri F et al. (2018). Inhibition of chloride intracellular channel 1 (CLIC1) as biguanide class-effect to impair human glioblastoma stem cell viability. *Front Pharmacol*. 9:899.
- Gessi M et al. (2015). Molecular heterogeneity characterizes glioblastoma with lipoblast/adipocyte-like cytology. *Virchows Arch*. 467:105-9.
- Gessi M et al. (2014). MYCN amplification predicts poor outcome for patients with supratentorial primitive neuroectodermal tumors of the central nervous system. *Neuro Oncol*. 16:924-32.
- Gessi M et al. (2013). Genome-wide DNA copy number analysis of desmoplastic infantile astrocytomas and desmoplastic infantile gangliogliomas. *J Neuropathol Exp Neurol*. 72:807-15.
- Gessi M et al. (2013). H3.3 G34R mutations in pediatric primitive neuroectodermal tumors of central nervous system (CNS-PNET) and pediatric glioblastomas: possible diagnostic and therapeutic implications? *J Neurooncol*. 112:67-72.
- Gielen GH et al. (2015). Genetic Analysis of Diffuse High-Grade Astrocytomas in Infancy Defines a Novel Molecular Entity. *Brain Pathol*. 25:409-17.
- Janik K et al (2019). A way to understand idiopathic senescence and apoptosis in primary glioblastoma cells – possible approaches to circumvent these phenomena. *BMC Cancer*. 19:923.

- Kakegawa S et al. (2020). Semi-comprehensive analysis of gene amplification in thymic malignant tumors using multiplex ligation-dependent probe amplification and fluorescence in situ hybridization. *Int J Clin Exp Pathol*. 13:1035-44.
- Lee SE et al. (2014). High level of CDK4 amplification is a poor prognostic factor in well-differentiated and dedifferentiated liposarcoma. *Histol Histopathol*. 29:127-38.
- Minarikova P et al. (2016). Prognostic Importance of Cell Cycle Regulators Cyclin D1 (CCND1) and Cyclin-Dependent Kinase Inhibitor 1B (CDKN1B/p27) in Sporadic Gastric Cancers. *Gastroenterol Res Pract*. 9408190.
- Monticone M et al. (2012). Identification of a novel set of genes reflecting different in vivo invasive patterns of human GBM cells. *BMC Cancer*. 12:358.
- Ooi A and Oyama T. (2018). Detection of CCND1 gene copy number variations using multiplex ligation-dependent probe amplification and fluorescence in situ hybridization methods. *Methods Mol Biol*. 1726:101-9.
- Ooi A et al. (2015). Semi-comprehensive analysis of gene amplification in gastric cancers using multiplex ligation-dependent probe amplification and fluorescence in situ hybridization. *Mod Pathol*. 28: 861-71.
- Oyama T et al. (2015). Overexpression and gene amplification of both ERBB2 and EGFR in an esophageal squamous cell carcinoma revealed by fluorescence in situ hybridization, multiplex ligation-dependent probe amplification and immunohistochemistry. *Pathol Int*. 65:608-13.
- Pokorna P et al. (2024). Real-World Performance of Integrative Clinical Genomics in Pediatric Precision Oncology. *Lab Invest*. 104:102161.
- Salvi S et al. (2014). Copy number analysis of 24 oncogenes: MDM4 identified as a putative marker for low recurrence risk in non muscle invasive bladder cancer. *Int J Mol Sci*. 15:12458-68.
- Schäfer N et al. (2019). Longitudinal heterogeneity in glioblastoma: moving targets in recurrent versus primary tumors. *J Transl Med*. 17:96.
- Stoczynska-Fidelus E et al. (2014). The failure in the stabilization of glioblastoma-derived cell lines: spontaneous in vitro senescence as the main culprit. *PLoS One*. 9:e87136.
- Tajiri R et al. (2014). Intratumoral heterogeneous amplification of ERBB2 and subclonal genetic diversity in gastric cancers revealed by multiple ligation-dependent probe amplification and fluorescence in situ hybridization. *Hum Pathol*. 45:725-34.
- Yamaura T et al. (2020). Genetic alterations in epidermal growth factor receptor-tyrosine kinase inhibitor-naïve non-small cell lung carcinoma. *Oncol Lett*. 19:4169-76.
- Zieba J et al. (2015). Sensitivity of neoplastic cells to senescence unveiled under standard cell culture conditions. *Anticancer Res*. 35:2759-68.

P175 product history	
Version	*Modification*
B2	One reference probe has been replaced.
B1	Six target probes have been replaced for the *AR*, *CCND2*, *ERBB2*, *FGFR1*, *PDGFRA,* and *RET* genes. One target probe for the *CCND1* gene has been removed. Several probes have been changed in length. In addition, 13 reference probes have been added and the data analysis method has been modified.
A3	Several probes have been changed in length.
A2	One target probe for *CDK4* gene has been replaced and one probe for *RET* gene has been changed in length.
A1	First release.

Implemented changes in the product description
Version B2-01 – 24 March 2025 (05P) - Product description rewritten and adapted to a new template and to a new product version (version number changed, changes in Table 1, Table 2 and Table 3). - Modifications in the content of Table 2, mainly regarding the content of probes in other probemixes. - Various minor textual or layout changes. - Exon numbering of the *ABL1*, *ERBB2,* and *AURKA* genes has been updated according to MANE select transcripts. - New reference added in 'Selected publications using P175 Tumour Gain' section. Version B1-04 – 10 January 2023 (04P) - Added information about possible small signal for BRAF p.V600E mutation probe on a sample with p.V600K mutation to P175 specific notes section and Tables 1 and 2. - Removed sample NA08035 from table of Positive control DNA samples. Version B1-03 – 29 March 2022 (04P) - Product description rewritten and adapted to a new template. - Several selected publications using probemix P175 Tumour Gain have been added. - Several minor textual changes throughout the document - Added information on additional positive samples on page 3. - Source of exon numbering updated to include LRG and/or NG information (when available).

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