

Product Description

SALSA® MLPA® Probemix P425-B2 Multiple Myeloma

To be used with the MLPA General Protocol.

Version B2

For complete product history see page 11.

Catalogue numbers:

- **P425-025R:** SALSA® MLPA® Probemix P425 Multiple Myeloma, 25 reactions.
- **P425-050R:** SALSA® MLPA® Probemix P425 Multiple Myeloma, 50 reactions.
- **P425-100R:** SALSA® MLPA® Probemix P425 Multiple Myeloma, 100 reactions.

SALSA® MLPA® Probemix P425 Multiple Myeloma (hereafter: P425 Multiple Myeloma) is to be used in combination with:

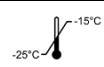

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P425-025R	P425-050R	P425-100R	
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 P425 Multiple Myeloma is a **research use only (RUO)** assay for the detection of deletions or duplications in the following chromosomal regions and target genes: 1p32-p12, 1q21-q23, 5q31, chr. 9, 12p13, 13q14-q22 (*RB1-DLEU2-DIS3*), 14q32 (*TRAF3*), chr. 15, 16q12 (*CYLD*), 16q23 (*WWOX*) and 17p13 (*TP53*). These regions and genes are suggested to be of prognostic relevance in multiple myeloma.

Multiple myeloma (MM) is a clonal B-cell disorder characterized by malignant proliferation of monoclonal plasma cells. MM cases present a common histological and morphological diagnosis, but enormous genetic and molecular complexity, as well as marked variations in clinical characteristics and in patient survival. Recent progress in molecular cytogenetics has improved our understanding of the pathogenesis of MM and has also provided reasoning for molecular sub-classification of MM. Primary cytogenetic abnormalities detected in the initial stages of MM include trisomies (commonly involving chromosomes 5, 7, 9, 11, 13 and 15) and immunoglobulin heavy chain (IgH) translocations. Upon disease progression, secondary genetic alterations take place, frequently including del17p or monosomy 17, del13q or monosomy 13, del1p and 1p gain. MLPA has been shown to be a reliable technique to detect copy number alterations in MM (Alpar et al. 2013; Boyle et al. 2014; Dragos et al. 2022). As balanced translocations also have a high importance in the prognostic classification of MM patients, MLPA and i-FISH are suggested to be applied as complementary techniques in this entity.

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: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>
 For NM_ mRNA reference sequences: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide>
 Locus Reference Genomic (LRG) database: <http://www.lrg-sequence.org/>
 Matched Annotation from NCBI and EMBL-EBI (MANE): <http://www.ncbi.nlm.nih.gov/refseq/MANE/>
 Tark – Transcript Archive: <http://tark.ensembl.org/>

Exon numbering

The *DAB1*, *CKS1B*, *CHD4*, *RB1*, *DIS3*, *TRAF3*, *CYLD*, *WWOX* and *TP53* exon numbering used in this P425-B2 Multiple Myeloma product description is the exon numbering derived from MANE project (release version 1.0) as indicated in Table 2. The *DAB1*, *DIS3*, *CYLD* and *TP53* 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. Please note that exon numbering for the same gene might be different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content

P425-B2 Multiple Myeloma contains 57 MLPA probes with amplification products between 129 and 499 nucleotides (nt). This includes 46 probes for the following chromosomal regions commonly deleted or gained in MM: 1p32-p12, 1q21-q23, 5q31, chr. 9, 12p13, 13q14-q22 (*RB1-DLEU2-DIS3*), 14q32 (*TRAF3*), chr. 15, 16q12-q23 (*CYLD-WWOX*) and 17p13 (*TP53*). In addition, 11 reference probes are included that target relatively copy number stable regions in various cancer types including MM. Complete probe sequences and the identity of the genes detected by the reference probes are available online and in Table 3 (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique

The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

MLPA technique validation

Internal validation 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.

Required specimens

Extracted DNA, which includes DNA derived from formalin-fixed, paraffin-embedded 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 multiple myeloma. 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 [P425 Multiple Myeloma product page](#) on our website.

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤ 0.10 . When this criterion is fulfilled, the following cut-off values for the final ratio (FR) of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	$0.80 < FR < 1.20$
Homozygous deletion	$FR = 0$
Heterozygous deletion	$0.40 < FR < 0.65$
Heterozygous duplication/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.

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

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *CDKN2C* gene. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: <http://dgv.tcag.ca/dgv/app/home>. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P425 specific note(s):

- The use of enrichment of CD138+ plasma cells increases the proportion of malignant myeloma cells in the sample and enhances sensitivity in detecting copy number aberrations compared to analysing samples with mixed cell populations (Boyle et al. 2014).

Limitations of the procedure

- In many tumour samples, genetic alterations in the *CYLD*, *DIS3*, *TP53* and *TRAF3* genes are small (point) mutations, none of which will not be detected by using SALSA MLPA Probemix P425 Multiple Myeloma.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the

corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in solid tumours with more chaotic karyotypes.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

COSMIC mutation database

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

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *TP53* exons 4b and 10 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. P425-B2 Multiple Myeloma

Length (nt)	MLPA probe	Chromosomal position (hg18) ^a			Location (hg18) in kb
		Reference	Other targets	Chromosome 1	
64-105	Control fragments – see table in probemix content section for more information				
129 ¥	Reference probe 20879-L25093	12q24			12-116,137
134	Reference probe 13867-L23116	16p13			16-008,765
138	TP53 probe 08304-L21074		17p13.1		17-007,518
144	CD27 probe 16210-L24732		12p13.31		12-006,430
148	RPE65 probe 04320-L14342			1p31.3	01-068,668
152	TRAF3 probe 18232-L24568		14q32.32		14-102,406
157	DIS3 probe 18233-L24283		13q22.1		13-072,248
160	Reference probe 16254-L19129	20q11			20-034,979
167 +	PBX1-area probe 18799-L24444			1q23.3	01-162,682
172 «	CDKN2C probe 14652-L16304			1p33	01-051,212
178	VAMP1 probe 16878-L24569		12p13.31		12-006,444
184	PCDHB10 probe 16214-L18467		5q31.3		05-140,552
190	DLEU2 probe 04020-L17532		13q14.3		13-049,554
196	LEPR probe 08867-L08923			1p31.3	01-065,809
202	Reference probe 03217-L24258	10q25			10-111,850
207	TRAF3 probe 18235-L24259		14q32.32		14-102,439
214	JAK2 probe 07444-L07092		9p24.1		09-005,041
221	CKS1B probe 16216-L19011			1q21.3	01-153,217
226 #	SLC25A2 probe 16218-L18471		5q31.3		05-140,663
236	Reference probe 09100-L24261	4q25			04-110,907
244	CKS1B probe 16220-L18473			1q21.3	01-153,214
250	RB1 probe 01784-L17337		13q14.2		13-047,835
256	TP53 probe 02376-L24733		17p13.1		17-007,519
263	NUF2 probe 16224-L18477			1q23.3	01-161,592
268	CYLD probe 16225-L18478		16q12.1		16-049,386
274	Reference probe 10708-L18855	6p12			06-052,023
280 +	PBX1-area probe 16226-L18479			1q23.3	01-162,602
286	FAF1 probe 02877-L18857			1p33	01-051,026
292	GABRB3 probe 10875-L11545		15q12		15-024,357
299	CHD4 probe 16228-L18481		12p13.31		12-006,586
306	COL11A1 probe 13242-L18856			1p21.1	01-103,177
315	Reference probe 06741-L24262	8q12			08-061,816
321 +	PBX1-area probe 16229-L18482			1q23.3	01-162,573
328	DPYD probe 18797-L24263			1p21.3	01-097,431
337	PBX1 probe 12517-L24268			1q23.3	01-163,083
344	PCDHGA11 probe 16230-L24269		5q31.3		05-140,781
351	WWOX probe 11972-L24270		16q23.1		16-076,691
358	CHD4 probe 16231-L24271		12p13.31		12-006,550
364	DAB1 probe 16232-L24272			1p32.2	01-057,253
372	Reference probe 05024-L24735	2q32			02-189,568
379	DIS3 probe 18237-L24734		13q22.1		13-072,234
386	WWOX probe 02305-L18859		16q23.1		16-077,016
391	TP53 probe 01587-L01159		17p13.1		17-007,515
400 j	TENT5C probe 18949-L24912			1p12	01-117,967
407	Reference probe 01237-L24913	10p14			10-012,019
414	PCDHAC1 probe 16234-L24914		5q31.3		05-140,287
421	COL5A1 probe 10394-L25152		9q34.3		09-136,837
427	NCAPD2 probe 16235-L18488		12p13.31		12-006,511
436 ‡	PLPP3 probe 18798-L24446			1p32.2	01-056,775
445	DAB1 probe 16237-L24275			1p32.2	01-058,122

Length (nt)	MLPA probe	Chromosomal position (hg18) ^a			
		Reference	Other targets	Chromosome 1	Location (hg18) in kb
453	RB1 probe 01799-L16912		13q14.2		13-047,949
461	PCDHA1 probe 16238-L24264		5q31.3		05-140,147
468	IGF1R probe 07607-L24266		15q26.3		15-097,300
474	CYLD probe 16239-L24267		16q12.1		16-049,334
483	PCDHB2 probe 16240-L18493		5q31.3		05-140,454
490	Reference probe 14909-L17745	18p11			18-013,724
499	Reference probe 09870-L15194	2p15			02-061,126

¥ Changed in version B2. Minor alteration, no change in sequence detected.

« 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.

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 RP11-480N10 (167 nt) and RP11-541J2 (321 and 280 nt) probes target a gene-poor region on 1q23.3 upstream of *PBX1* gene renamed to *PBX1*-area.

‡ *TENT5C* gene name has been changed (*FAM46C* previously) according to latest HGNC nomenclature.

‡ *PLPP3* gene name has been changed (*PPAP2B* previously) according to latest HGNC nomenclature.

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 hybridization 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)	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
1p deletions					
Deletion of 1p is commonly described in MM and loss of 1p has been shown to correlate with unfavourable outcome for MM patients. Deletion of 1p31-32 (harbouring the <i>FAF1</i> , <i>CDKN2C</i> , <i>PLPP3</i> [‡] and <i>DAB1</i> genes) has been shown to be an independent prognostic factor in MM (McAvoy et al. 2008; Leone et al. 2008; Chng et al. 2010; Walker et al. 2010; Perrot et al. 2019). In addition, loss of 1p21 is reported to be an adverse prognostic factor in myeloma (Chang et al. 2007; Chang et al. 2010). Fine mapping of chromosome 1p deletions in MM identifies <i>TENT5C</i> [‡] at 1p12 associated with adverse survival (Boyd et al. 2011), and moreover, <i>TENT5C</i> [‡] is mutated in ~13% of MM patients (Chapman et al. 2011).					
286	02877-L18857	FAF1	1p33	GGACCTGCATTT-AATCCAGCAAGT	0.2 Mb
172 «	14652-L16304	CDKN2C	1p33	TGCTGGAGTTTC-AAGCTGATGTTA	5.6 Mb
436 ‡	18798-L24446	PLPP3	1p32.2	AGCACCATCAAG-CCTTACCACCGA	0.5 Mb
364	16232-L24272	DAB1 , exon 12 (14)	1p32.2	CACAAACTGTTA-TGCCCTTTGCCAG	0.9 Mb
445	16237-L24275	DAB1 , upstream (2)	1p32.2	GACGATTCCTGA-CTCGTGGCCCCG	7.7 Mb
196	08867-L08923	LEPR	1p31.3	TGCTTTCGGAGT-GAGCAAGATAGA	2.9 Mb
148	04320-L14342	RPE65	1p31.3	CAGAATCAGGAG-ATAAGCAGGCTT	28.8 Mb
328	18797-L24263	DPYD	1p21.3	TGGGATGGACAG-AGTCCAGCTACT	5.7 Mb
306	13242-L18856	COL11A1	1p21.1	TTTCAGGGTGAA-ATTGGTGAGCCG	14.8 Mb
400 ‡	18949-L24912	TENT5C	1p12	CAGCCAGAACAT-CCCCTGAAGATG	35.2 Mb
1q amplifications and gains					
1q amplification is associated with poor survival and is considered as a high risk cytogenetic abnormality (Schmidt et al. 2021). Amplifications or gains of 1q21 (<i>CKS1B</i>) and 1q23.3 are associated with rapid disease progression and are signs of poor prognosis in MM (Shaughnessy et al. 2005; Hanamura et al. 2006; Avet-Loiseau et al. 2009; Walker et al. 2010; Shah V et al. 2018).					
244	16220-L18473	CKS1B , exon 1	1q21.3	TTTGGCCGCTGA-GGGCACAAGGAA	3.1 kb
221	16216-L19011	CKS1B , exon 2	1q21.3	TTCTGTTACAGA-CATGTGATGCTG	8.4 Mb
263	16224-L18477	NUF2	1q23.3	AGCAGAGGACTC-CTATGCTAAGAT	981.5 kb
321 +	16229-L18482	PBX1 -area	1q23.3	GAACATCCCATA-ATGGATTGAAG	28.5 kb
280 +	16226-L18479	PBX1 -area	1q23.3	CCCTCATCCCTA-CCCTAGAGTCAC	80.1 kb

Length (nt)	MLPA probe	Gene, exon ^a	Location (hg18)	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
167 +	18799-L24444	PBX1 -area	1q23.3	CTCTGAGAATCT-CCTCAAGAAGCC	400.8 kb
337	12517-L24268	PBX1	1q23.3	ACTGGAGGTCGA-AGCAATCAGCAA	-
5q amplifications and gains					
Amplification of 5q31.3 is reported to be a favourable prognostic factor in MM (Avet-Loiseau et al. 2009), however, this marker is not used for routine prognostication yet, as controversial findings appear (Tapper et al. 2011). Moreover, the most sensitive and specific combination for the detection of hyperdiploidy in MM is suggested to be copy number detection of chromosomes 5, 9 and 15 (Wuilleme et al. 2005).					
461	16238-L24264	PCDHA1	5q31.3	GTATACAGAGTC-CACTTGTAGAG	140.6 kb
414	16234-L24914	PCDHAC1	5q31.3	GGGACTGTGTTA-TTCCGAGTTCAA	167.1 kb
483	16240-L18493	PCDHB2	5q31.3	ATCCCAGTATCA-GCGAGATACGGG	97.8 kb
184	16214-L18467	PCDHB10	5q31.3	TGGCTGTAACCA-ACTAGGAAATAA	110.4 kb
226 #	16218-L18471	SLC25A2	5q31.3	ACAGCAGGAAGA-TGATGATGAAAC	118.2 kb
344	16230-L24269	PCDHGA11	5q31.3	CACAACCAACCA-GCTCGAGAAACC	-
Chromosome 9 gains					
The most sensitive and specific combination for the detection of hyperdiploidy in MM is suggested to be copy number detection of chromosomes 5, 9 and 15 (Wuilleme et al. 2005). Hyperdiploidy in MM is suggested to be associated with favourable prognosis (Fonseca et al. 2003).					
214	07444-L07092	JAK2	9p24.1	CTAACACTGCCA-TCCCAAGACATT	131.8 Mb
421	10394-L25152	COL5A1	9q34.3	TCTGACTCTGTT-TTCAGGGTGACC	-
12p deletions					
Deletion of 12p is suggested to be an unfavourable prognostic factor in MM (Avet-Loiseau et al. 2009; Li et al. 2015).					
144	16210-L24732	CD27	12p13.31	CCATCACTGCCA-ATGCTGAGTGTG	14.4 kb
178	16878-L24569	VAMP1	12p13.31	CTCCTGTTCTGA-GGAAGTGGGGCT	66.8 kb
427	16235-L18488	NCAPD2	12p13.31	GCACAGATCCTA-GGAAGTCTGTTC	39.2 kb
358	16231-L24271	CHD4 , exon 40	12p13.31	ACCACCTCCACC-GCTGAGCAGTGA	35.7 kb
299	16228-L18481	CHD4 , exon 2	12p13.31	ATATGGATGCAC-TTTTGAACAACA	-
13q deletions					
Loss of 13q is detected in ~50% of MM cases and the minimal region of deletion harbours the <i>RB1</i> , <i>DLEU1/2</i> and <i>DIS3</i> genes (Elneaei et al. 2003). Deletion of <i>RB1</i> , alongside abnormalities of chr.13, is reported to be a sign of poor prognosis in MM (Zojer et al. 2000; Shaughnessy et al. 2003). Deletions of <i>DIS3</i> are detected in ~45% of MM patients and <i>DIS3</i> is also frequently mutated in MM (Chapman et al. 2011; Morgan et al. 2012).					
250	01784-L17337	RB1 , exon 8	13q14.2	GCGAGGTCAGAA-CAGGAGTGCACG	114.1 kb
453	01799-L16912	RB1 , exon 26	13q14.2	AGAGTCCAAATT-TCAGCAGAAACT	1.6 Mb
190	04020-L17532	DLEU2	13q14.3	CGCATGCGTAAA-AATGTGCGGAAA	22.7 Mb
379	18237-L24734	DIS3 , exon 17 (18)	13q22.1	TGTATGATGCAA-GCTGTGTACTTC	14.0 kb
157	18233-L24283	DIS3 , exon 5 (6)	13q22.1	GTAAGCTACAGC-AAGGCATAAAAT	-
TRAF3 deletions, at 14q32.32					
Deletions of <i>TRAF3</i> are detected in ~15% of MM cases, while <i>TRAF3</i> is also commonly mutated in MM, and this may play an important role in response to proteasome inhibitor bortezomib therapy (Keast et al. 2007; Du et al. 2011).					
152	18232-L24568	TRAF3 , exon 3	14q32.32	GAGGACAAGTAC-AAGTGTGAGAAG	33.0 kb
207	18235-L24259	TRAF3 , exon 11	14q32.32	AAGAGCAGCGTG-GAGTCCCTCCAG	-
Chromosome 15 gains					
The most sensitive and specific combination for the detection of hyperdiploidy in MM is suggested to be copy number detection of chromosomes 5, 9 and 15 (Wuilleme et al. 2005). Hyperdiploidy in MM is suggested to be associated with favourable prognosis (Fonseca et al. 2003).					
292	10875-L11545	GABRB3	15q12	CACCACTTTGTT-TCTTTTCTAGGG	72.9 Mb
468	07607-L24266	IGF1R	15q26.3	CATGGTAGCCGA-AGATTTACAGT	-
16q12 and 16q23 deletions					
Loss of the 16q arm is detected in ~40% of MM patient samples (Walker et al. 2010) and deletion of 16p12 (<i>CYLD</i>) and 16q23 (<i>WWOX</i>) is suggested to be associated with adverse clinical outcome in MM (Jenner et al. 2007).					

Length (nt)	MLPA probe	Gene, exon ^a	Location (hg18)	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
474	16239-L24267	CYLD , exon 2	16q12.1	TGGTTCTACACA-GCCACCCGGAGT	51.4 kb
268	16225-L18478	CYLD , exon 18 (19)	16q12.1	AGGCTGAATCAT-AAATATAACCCA	27.3 Mb
351	11972-L24270	WWOX , exon 1	16q23.1	AACCACCAAGGA-CGGCTGGGTTTA	325.1 kb
386	02305-L18859	WWOX , exon 7	16q23.1	GGCCTGGAGACC-ACCTTTCAAGTG	-
TP53 deletions, at 17p13.1					
Several studies have shown that 17p13 deletions, targeting the <i>TP53</i> tumour suppressor gene, are associated with poor clinical outcome in MM (Drach et al. 1998; Fonseca et al. 2003; Chang et al. 2005; Avet-Loiseau et al. 2007; Walker et al. 2010; Boyd et al. 2011). According to multiple risk stratification approaches, del 17p is a determinant of stage III/ high risk disease (Palumbo et al. 2015; Chng et al. 2014; Mikhael et al. 2013)					
391	01587-L01159	TP53 , exon 10	17p13.1	TTCCGAGAGCTG-AATGAGGCCTTG	3.1 kb
138	08304-L21074	TP53 , exon 8 (7)	17p13.1	CTGTCCTGGGAG-AGACCGGCGCAC	1.4 kb
256	02376-L24733	TP53 , exon 5 (4b)	17p13.1	CAAGATGTTTTG-CCAACCTGGCCAA	-

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

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

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

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 RP11-480N10 (167 nt) and RP11-541J2 (321 and 280 nt) probes target a gene-poor region on 1q23.3 upstream of *PBX1* gene renamed to PBX1-area.

‡ *TENT5C* gene name has been changed (*FAM46C* previously) according to latest HGNC nomenclature.

‡ *PLPP3* gene name has been changed (*PPAP2B* previously) according to latest HGNC nomenclature.

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
499	09870-L15194	PEX13	2p15	TGAGGATGACCA-TGTAGTTGCCAG	02-061,126
372	05024-L24735	COL3A1	2q32	GGTGTCCAGGA-GCTAAAGCGGAA	02-189,568
236	09100-L24261	CFI	4q25	TGTGTGCAACTA-ACAGGAGAAGCT	04-110,907
274	10708-L18855	PKHD1	6p12	TCTCAAGCTGAT-TCTGGAACGGCT	06-052,023
315	06741-L24262	CHD7	8q12	CACCTCAGTGAA-GTGAAGCACAGG	08-061,816
407	01237-L24913	UPF2	10p14	TGCCATTCCTTT-GCATCTCAAAG	10-012,019
202	03217-L24258	ADD3	10q25	AAGAGAGATATT-TTGACCGCATCA	10-111,850
129	20879-L25093	NOS1	12q24	ACTGCTGAACCT-TTCCTCTGGGAC	12-116,137
134	13867-L23116	ABAT	16p13	ACTTTGTGGAGA-AGCTCCGGCAGT	16-008,765
490	14909-L17745	RNMT	18p11	TACAATGAACTT-CAGGAAGTTGGT	18-013,724
160	16254-L19129	SAMHD1	20q11	GGCGTCTGAAA-ACAAAAGCTTCC	20-034,979

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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P425 product history	
Version	Modification
B2	One probe has a change in length, but no change in the sequence detected.
B1	New probes were added to <i>FAM46C</i> , <i>DIS3</i> , <i>TRAF3</i> , chr.9 and chr.15. Moreover, several reference probes were replaced.
A1	First release.

Implemented changes in the product description


Version B2-02 – 10 December 2025 (05P)

- Product description rewritten and adapted to a new template.
- Small changes of probe lengths for three probes in Table 1 and 2 in order to better reflect the true lengths of the amplification products (400, 453, 468 nt).
- FAM46C renamed to *TENT5C* (probe 18949-L24912) throughout the document.
- Three probes in gene-poor region on 1q23.3 upstream of *PBX1* gene renamed to PBX1-area.
- Removed the “« Probe located in or near a GC-rich region...” remark in Table 1 and 2 for CYLD probe 16239-L24267 at 474 nt.
- Added a separate table for Reference probes.
- Positive samples section replaced with referral to P425 product page.
- Exon numbering of the *DAB1*, *DIS3*, *CYLD* and *TP53* genes has been changed.
- Added new references in Table 2 and ‘References’ section.
- Added new references in section ‘Selected publications using P425 Multiple Myeloma’.

Version B2-01 – 30 July 2020 (02P)

- Product description rewritten and adapted to a new template.
- Various minor textual and layout changes.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Several new references added on pages 8-9.

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

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