Product Description SALSA® MLPA® Probemix P425-B2 Multiple Myeloma

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, one probe has a change in length, but no change in the sequence detected. For complete product history see page 10.

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.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information: The SALSA MLPA Probemix 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 MM.

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 and Boyle et al. 2014). 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 complimentary techniques in this entity.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering: The exon numbering used in this P425-B2 Multiple Myeloma product description is the exon numbering from the LRG_301 for *CDH1*; NG_029028.1 for *CKS1B;* LRG_491 for *CYLD;* NG_046914.2 for *DAP1;* NM_014953.5 for *DIS3;* LRG_517 for *RB1;* LRG_321 for *TP53;* LRG_229 for *TRAF3* and NG_011698.1 for *WWOX*. The exon numbering, LRG_ sequence, NG_ sequence and NM_ sequences have

been retrieved on 06/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix 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 are available online (www.mlpa.com) and the identity of the genes detected by the reference probes is available in Table 2.

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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment 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.mlpa.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens: Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of multiple myeloma. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Samples from the Coriell Institute and Leibniz Institute DSMZ have been tested with this P425-B2 probemix at MRC Holland and can be used as a positive control samples to detect several copy number alterations as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P425-B2	Expected CNA
NA00803	Coriell Institute	1q23.3	*NUF2, RP11s* and *PBX1*	Heterozygous deletion
NA06038	Coriell Institute	1q23.3	*PBX1*	Heterozygous deletion

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P425-B2	Expected CNA
NA10989	Coriell Institute	9p24.1	*JAK2*	Heterozygous deletion
NA07981	Coriell Institute	12p13.31	*CD27, VAMP1, NCAPD2 and CHD4*	Heterozygous triplication / Homozygous duplication
NA14164	Coriell Institute	13q14.2-q22.1	*RB1, DLEU2* and *DIS3*	Heterozygous deletion
NA05832	Coriell Institute	13q14.2-q22.1	*RB1, DLEU2* and *DIS3*	Heterozygous duplication
NA03184	Coriell Institute	15q12-q26.3	*GABRB3* and *IGF1R*	Heterozygous duplication
NA20375	Coriell Institute	15q12	*GABRB3*	Heterozygous deletion
NA03255	Coriell Institute	15q26.3	*IGF1R*	Heterozygous deletion
NA09687	Coriell Institute	16q23.1	*WWOX*	Heterozygous duplication

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P425-B2 Multiple Myeloma probemix.

Data analysis: Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.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 criteria is fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

Please note that these above mentioned dosage quotients are only valid for germline testing. Dosage quotients 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 mosaic 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

P425 specific notes:

- 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 most populations, the major cause of genetic defects in the chromosomal regions included in this probemix are small (point) mutations, most 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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 samples 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 SNPs and unusual results (e.g., a deletion of *TP53* exons 4b and 10 but not exon 7) to MRC Holland: info@mlpa.com.

This probemix was developed at MRC Holland in collaboration with Institute of Cancer Research (ICR), London.

Table 1. SALSA MLPA Probemix P425-B2 Multiple Myeloma

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	Chr. 1	Other targets	Location (hg18) in kb
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	**RP11-480N10** probe 18799-L24444		1**q**23.3		01-162.682
172 «	**CDKN2C** probe 14652-L16304		1p33		01-051.212
178	**VAMP1** probe 16878-L24569			12p13.3	12-006.444
184	**PCDHB10** probe 16214-L18467			5q31.3	05-140.552
190	**DLEU2** probe 04020-L17532			13q14.2	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		1**q**21.3		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		1**q**21.3		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		1**q**23.3		01-161.592
268	**CYLD** probe 16225-L18478			16q12.1	16-049.386
274	Reference probe 10708-L18855	6p12			06-052.023
280	**RP11-541J2** probe 16226-L18479		1**q**23.3		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	**RP11-541J2** probe 16229-L18482		1**q**23.3		01-162.573
328	**DPYD** probe 18797-L24263		1p21.3		01-097.431
337	**PBX1** probe 12517-L24268		1**q**23.3		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			12p13.3	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
399	**FAM46C** 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
454	**RB1** probe 01799-L16912			13q14.2	13-047.949
461	**PCDHA1** probe 16238-L24264			5q31.3	05-140.147
467	**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

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	Chr. 1	Other targets	Location (hg18) in kb
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.
‡ *PLPP3* gene name has been changed (*PPAP2B* previously) as we have adopted the novel HUGO name for this gene.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Table 2. P425 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location (hg18) / Exon	Partial sequence (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 *FAF1, CDKN2C, PPAP2B* and *DAB1* 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). 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 *FAM46C* at 1p12 associated with adverse survival (Boyd et al. 2011), and moreover, *FAM46C* 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**	1p32.2; exon 14	CACAAACTGTTA-TGCCTTTGCCAG	0.9 Mb
445	16237-L24275	**DAB1**	1p32.2; exon 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	28.7 Mb
328	18797-L24263	**DPYD**	1p21.3	TGGGATGGACAG-AGTCCAGCTACT	5.7 Mb
306	13242-L18856	**COL11A1**	1p21.1	TTTCAGGGTGAA-ATTGGTGAGCCG	14.8 Mb
399	18949-L24912	**FAM46C**	1p12	CAGCCAGAACAT-CCCCTGAAGATG	3.5 Mb To ***CKS1B*** gene
1q amplifications and gains Amplifications or gains of 1q21 *(CKS1B)* 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). *Please note that RP11-541J2 and RP11-480N10 are not gene names but the codes for BAC clones that span this particular gene-poor region. The nearest gene, starting at a distance of approximately 400 kb telomeric from the 167 nt probe, is *PBX1*.					
244	16220-L18473	**CKS1B**	1q21.3; exon 1	TTTGGCCGCTGA-GGGCACAAGGAA	3.1 **kb**
221	16216-L19011	**CKS1B**	1q21.3; exon 2	TTCTGTTACAGA-CATGTCATGCTG	8.4 Mb
263	16224-L18477	**NUF2**	1q23.3	AGCAGAGGACTC-CTATGCTAAGAT	1.0 Mb
321 *	16229-L18482	RP11-541J2	1q23.3	GAACATCCCATA-ATGGATTTGAAG	28.5 **kb**
280 *	16226-L18479	RP11-541J2	1q23.3	CCCTCATCCCTA-CCCTAGAGTCAC	80.1 **kb**
167 *	18799-L24444	RP11-480N10	1q23.3	CTCTGAGAATCT-CCTCAAGAAGCC	0.4 Mb
337	12517-L24268	**PBX1**	1q23.3	ACTGGAGGTCGA-AGCAATCAGCAA	-
Reference probes on 2p, 2q and 4q					
499	09870-L15194	PEX13	2p15	TGAGGATGACCA-TGTAGTTGCCAG	128 Mb
372	05024-L24735	COL3A1	2q32	GGTGTTCCAGGA-GCTAAAGGCGAA	-
236	09100-L24261	CFI	4q25	TGTGTGCAACTA-ACAGGAGAAGCT	-
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 controversary 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-CACTTGTTAGAG	0.1 Mb

Length (nt)	SALSA MLPA probe	Gene	Location (hg18) / Exon	**Partial** sequence (24 nt adjacent to ligation site)	Distance to next probe
414	16234-L24914	**PCDHAC1**	5q31.3	GGGACTGTGTTA-TTCCGAGTTCAA	0.2 Mb
483	16240-L18493	**PCDHB2**	5q31.3	ATCCCAGTATCA-GCGAGATACGGG	0.1 Mb
184	16214-L18467	**PCDHB10**	5q31.3	TGGCTGTAACCA-ACTAGGAAATAA	0.1 Mb
226 #	16218-L18471	**SLC25A2**	5q31.3	ACAGCAGGAAGA-TGATGATGAAAC	0.1 Mb
344	16230-L24269	**PCDHGA11**	5q31.3	CACAACCAACCA-GCTCGAGAAACC	-
Reference probes on 6p and 8q					
274	10708-L18855	PKHD1	6p12	TCTCAAGCTGAT-TCTGGAACGGCT	-
315	06741-L24262	CHD7	8q12	CACCTCAGTGAA-GTGAAGCACAGG	-
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	-
Reference probes on 10p and 10q.					
407	01237-L24913	UPF2	10p14	TGCCATTCCTTT-GCATCTCAAAAG	99.8 Mb
202	03217-L24258	ADD3	10q25	AAGAGAGATATT-TTGACCGCATCA	-
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**	12p13.31; exon 40	ACCACCTCCACC-GCTGAGCAGTGA	35.7 **kb**
299	16228-L18481	**CHD4**	12p13.31; exon 2	ATATGGATGCAC-TTTTGAACAACA	109.5 Mb To ***NOS1*** gene
Reference probe on 12q.					
129	20879-L25093	NOS1	12q24	ACTGCTGAACCT-TTCCTCTGGGAC	-
13q deletions Loss of 13q is detected in ~50% of MM cases and the minimal region of deletion harbours *RB1, DLEU1/2* and *DIS3* genes (Elneaei et al. 2003). Deletion of *RB1*, 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 *DIS3* are detected in ~45% of MM patients and *DIS3* is also frequently mutated in MM (Chapman et al. 2011; Morgan et al. 2012).					
250	01784-L17337	**RB1**	13q14.2; exon 8	GCGAGGTCAGAA-CAGGAGTGCACG	0.1 Mb
454	01799-L16912	**RB1**	13q14.2; exon 26	AGAGTCCAAATT-TCAGCAGAAACT	1.6 Mb
190	04020-L17532	**DLEU2**	13q14.3	CGCATGCGTAAA-AATGTCGGGAAA	22.7 Mb
379	18237-L24734	**DIS3**	13q22.1; exon 18	TGTATGATGCAA-GCTGTGTACTTC	14.0 **kb**
157	18233-L24283	**DIS3**	13q22.1; exon 6	GTAAGCTACAGC-AAGGCATAAAAT	-
***TRAF3* deletions**, at 14q32.32 Deletions of *TRAF3* are detected in ~15% of MM cases, while *TRAF3* 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**	14q32.32; exon 3	GAGGACAAGTAC-AAGTGTGAGAAG	33.0 **kb**
207	18235-L24259	**TRAF3**	14q32.32; exon 11	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
467	07607-L24266	**IGF1R**	15q26.3	CATGGTAGCCGA-AGATTTCACAGT	-
Reference probe on 16p					
134	13867-L23116	ABAT	16p13	ACTTTGTGGAGA-AGCTCCGGCAGT	40.5 Mb To ***CYLD*** gene
16q12 and 16q23 deletions Loss of the 16q arm is detected ~40% of MM patient samples (Walker et al. 2010) and deletion of 16p12 *(CYLD)* and 16q23 *(WWOX)* is suggested to be associated with adverse clinical outcome in MM (Jenner et al. 2007).					
474 «	16239-L24267	**CYLD**	16q12.1; exon 2	TGGTTCTACACA-GCCACCCGGAGT	51.4 **kb**

Length (nt)	SALSA MLPA probe	Gene	Location (hg18) / Exon	**Partial** sequence (24 nt adjacent to ligation site)	Distance to next probe
268	16225-L18478	**CYLD**	16q12.1; exon 19	AGGCTGAATCAT-AAATATAACCCA	27.3 Mb
351	11972-L24270	**WWOX**	16q23.1; exon 1	AACCACCAAGGA-CGGCTGGGTTTA	0.3 Mb
386	02305-L18859	**WWOX**	16q23.1; exon 7	GGCCTGGAGACC-ACCTTTCAAGTG	-
***TP53* deletions**, at 17p13.1 Several studies have shown that 17p13 deletions, targeting the *TP53* 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**	17p13.1; exon 10	TTCCGAGAGCTG-AATGAGGCCTTG	3.1 **k**b
138	08304-L21074	**TP53**	17p13.1; exon 7	CTGTCCTGGGAG-AGACCGGCGCAC	1.4 **k**b
256	02376-L24733	**TP53**	17p13.1; exon 4b	CAAGATGTTTTG-CCAACTGGCCAA	-
Reference probes on 18p and 20					
490	14909-L17745	RNMT	18p11	TACAATGAACTT-CAGGAAGTTGGT	-
160	16254-L19129	SAMHD1	20q11	GGCGTCCTGAAA-ACAAAAGCTTCC	-

« This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

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

‡ *PLPP3* gene name has been changed (*PPAP2B* previously) as we have adopted the novel HUGO name for this gene.

a) See above section on exon numbering for more information.

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

Related SALSA MLPA probemixes

- P037 CLL-1 and P038 CLL-2: Contains 8 and 7 probes, respectively, for the 13q region *(RB1-DLEU1/2/7).*
- P056 TP53: Contains probes for each exon of *TP53* gene.
- P063 FHIT-WWOX: Contains probes for each exon of *WWOX* gene.

References

- Avet-Loiseau H et al. (2007). Genetic Abnormalities and Survival in Multiple Myeloma: The Experience of the Intergroupe Francophone Du Myélome. *Blood.* 109:3489-95.
- Avet-Loiseau H et al. (2009). Prognostic Significance of Copy-Number Alterations in Multiple Myeloma. *J Clin Oncol.* 27:4585-90.
- Binder M et al (2017). Prognostic Implications of Abnormalities of Chromosome 13 and the Presence of Multiple Cytogenetic High-Risk Abnormalities in Newly Diagnosed Multiple Myeloma. *Blood Cancer J.* 7:e600.
- Boyd KD et al. (2011). Mapping of Chromosome 1p Deletions in Myeloma Identifies FAM46C at 1p12 and CDKN2C at 1p32.3 as Being Genes in Regions Associated With Adverse Survival. *Clin Cancer Res*. 17:7776-84.
- Chng WJ et al. (2014). IMWG Consensus on Risk Stratification in Multiple Myeloma. *Leukemia.* 28:269-77.
- Drach J et al. (1998). Presence of a p53 Gene Deletion in Patients With Multiple Myeloma Predicts for Short Survival After Conventional-Dose Chemotherapy. *Blood.* 92:802-9.
- Du J et al. (2011). Polymorphisms of Nuclear factor-κB Family Genes Are Associated With Development of Multiple Myeloma and Treatment Outcome in Patients Receiving Bortezomib-Based Regimens. *Haematologica.* 96:729-37.
- Elneaei MO et al. (2003). Gene Abnormalities in Multiple Myeloma; The Relevance of TP53, MDM2, and CDKN2A. *Haematologica*. 88:529-37.
- Chang H et al. (2005). p53 Gene Deletion Detected by Fluorescence in Situ Hybridization Is an Adverse Prognostic Factor for Patients With Multiple Myeloma Following Autologous Stem Cell Transplantation. *Blood.* 105:358-60.

- Chang H et al. (2007). Chromosome 1p21 Deletion Is a Novel Prognostic Marker in Patients With Multiple Myeloma. *Br J Haematol*. 139:51-54.
- Chang H et al. (2010). 1p21 Deletions Are Strongly Associated With 1q21 Gains and Are an Independent Adverse Prognostic Factor for the Outcome of High-Dose Chemotherapy in Patients With Multiple Myeloma. *Bone Marrow Transplant*. 45:117-21.
- Chapman MA et al. (2011). Initial Genome Sequencing and Analysis of Multiple Myeloma. *Nature*. 471:467-72.
- Chng WJ et al. (2010). Correlation Between Array-Comparative Genomic Hybridization-Defined Genomic Gains and Losses and Survival: Identification of 1p31-32 Deletion as a Prognostic Factor in Myeloma. *Leukemia*. 24:833-42.
- Fonseca R et al. (2003). Clinical and Biologic Implications of Recurrent Genomic Aberrations in Myeloma. *Blood.* 101:4569-75.
- Hanamura L et al. (2006). Frequent Gain of Chromosome Band 1q21 in Plasma-Cell Dyscrasias Detected by Fluorescence in Situ Hybridization: Incidence Increases From MGUS to Relapsed Myeloma and Is Related to Prognosis and Disease Progression Following Tandem Stem-Cell Transplantation. *Blood.* 108:1724-32.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Jenner MW et al. (2007). Gene Mapping and Expression Analysis of 16q Loss of Heterozygosity Identifies WWOX and CYLD as Being Important in Determining Clinical Outcome in Multiple Myeloma. *Blood.* 110:3291-300.
- Keast JJ et al. (2007). Promiscuous Mutations Activate the Noncanonical NF-kappaB Pathway in Multiple Myeloma. *Cancer Cell.* 12:131-44.
- Leone PE et al. (2008). Deletions of CDKN2C in Multiple Myeloma: Biological and Clinical Implications. *Clin Cancer Res.* 14:6033-41.
- Li F et al. (2015). Heterogeneous Chromosome 12p Deletion Is an Independent Adverse Prognostic Factor and Resistant to Bortezomib-Based Therapy in Multiple Myeloma. *Oncotarget*. 6:9434-44.
- McAvoy S. et al. (2008). Disabled-1 Is a Large Common Fragile Site Gene, Inactivated in Multiple Cancers. *Genes Chromosomes Cancer.* 47:165-74.
- Mikhael JR et al. (2013). Management of Newly Diagnosed Symptomatic Multiple Myeloma: Updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) Consensus Guidelines 2013. *Mayo Clin Proc.* 88:360-76.
- Morgan G et al. (2012). The Genetic Architecture of Multiple Myeloma. *Nat Rev Cancer*. 12:335-48.
- Palumbo A et al. (2015). Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. *J Clin Oncol.* 33:2863-9.
- 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.
- Shaughnessy J et al. (2003). Continuous Absence of Metaphase-Defined Cytogenetic Abnormalities, Especially of Chromosome 13 and Hypodiploidy, Ensures Long-Term Survival in Multiple Myeloma Treated With Total Therapy I: Interpretation in the Context of Global Gene Expression. *Blood.* 101:3849-56.
- Shaughnessy J et al. (2005). Amplification and Overexpression of CKS1B at Chromosome Band 1q21 Is Associated With Reduced Levels of p27Kip1 and an Aggressive Clinical Course in Multiple Myeloma. *Hematology.* 10:117-26.
- Tapper W et al. (2011). Heterogeneity in the Prognostic Significance of 12p Deletion and Chromosome 5 Amplification in Multiple Myeloma. *J Clin Oncol.* 29:e37-9.
- 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.
- Walker BA et al. (2010). A compendium of myeloma-associated chromosomal copy number abnormalities and their prognostic value. *Blood*. 116:56-65.
- Wuilleme S et al. (2005). Ploidy, as Detected by Fluorescence in Situ Hybridization, Defines Different Subgroups in Multiple Myeloma. *Leukemia.* 19:275-8.
- Zojer N et al. (2000). Deletion of 13q14 Remains an Independent Adverse Prognostic Variable in Multiple Myeloma Despite Its Frequent Detection by Interphase Fluorescence in Situ Hybridization. *Blood*. 95:1925-30.

Selected publications using SALSA MLPA Probemix P425 Multiple Myeloma

- Alpar D et al. (2013). Multiplex ligation-dependent probe amplification and Fluorescence In Situ Hybridization are complementary techniques to detect cytogenetic abnormalities in multiple myeloma. *Genes Chromosomes Cancer*. 52:785-93.
- Boyle EM et al. (2015). A molecular diagnostic approach able to detect the recurrent genetic prognostic factors typical of presenting myeloma. *Genes Chromosomes Cancer*. 54:91-8.
- Gupta R et al. (2018). Nucleic acid based risk assessment and staging for clinical practice in multiple myeloma. *Ann Hematol.* 97:2447-54.
- Jones JR et al. (2019). Clonal evolution in myeloma: the impact of maintenance lenalidomide and depth of response on the genetics and sub-clonal structure of relapsed disease in uniformly treated newly diagnosed patients. Haematologica. 104:1440-50.
- Kosztolányi S et al. (2018). High-throughput copy number profiling by digital multiplex ligation-dependent probe amplification in multiple myeloma*. J Mol Diagn.* 20:777-88.
- Shah V et al. (2018). Prediction of outcome in newly diagnosed myeloma: a meta-analysis of the molecular profiles of 1905 trial patients. *Leukemia*. 32:102-10.
- Shah V et al. (2018). Sub-clonal TP53 copy number is associated with prognosis in multiple myeloma. *Blood*. 132:2465-9.
- Sommaluan S et al. (2017). The utilization of karyotyping, iFISH, and MLPA for the detection of recurrence genetic aberrations in multiple myeloma. *Asian Pac J Cancer Prev*. 18:3135-42.
- Zang M et al. (2015). Detection of recurrent cytogenetic aberrations in multiple myeloma: A comparison between MLPA and iFISH. *Oncotarget.* 6:34276-87.

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 *FAM46C, DIS3, TRAF3*, chr.9 and chr.15. Moreover, several reference probes were replaced.
A1	First release.

Implemented changes in the product description

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.

Version 08 – 07 December 2018 (T08)
- For uniformity, the chromosomal positions and bands in this document are now all based on hg18 (NCBI36).
- New references added on page 1.
- Several minor textual changes throughout the document.

Version 07 – 27 March 2018 (T08)
- Gene name adapted for *DLEU2*, on page 1 and in Table 2.
- New reference added on page 1.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 06 – 12 April 2017 (T08)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Small changes of probe lengths in Table 1 and Table 2 in order to better reflect the true lengths of the amplification products.
- New reference added on page 1.
- Various minor textual and layout changes.
- Ligation sites of the probes targeting the *DIS3* and *WWOX* genes are updated according to new versions of the NM_reference sequence.

Version 05 – 15 April 2016 (T08)
- *PLPP3* gene name has been changed (*PPAP2B* previously) as we have adopted the novel HUGO name for this gene.

Version 04 (T06)
- New reference added on page 1 for the probemix.
- Minor textual corrections on page 1.
- Updated link for "Database of Genomic Variants".
- "Peak area" replaced with "peak height".

Version 03 (T03)
- Product description adapted to a new product version (version number changed, lot number added, new pictures included).
- Various textual changes on page 1 & 2 and in table 2.

Version 02 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 01 (T01)
- Not applicable, new product description.

More information: www.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mlpa.com (information & technical questions); order@mlpa.com (orders)
Phone	+31 888 657 200