

Product Description

NXtec™ D006-A1 Multiple Myeloma

To be used with the digitalMLPA NXtec Protocol.

Version A1

First release.

Catalogue numbers

- **D006-025R:** NXtec D006 Multiple Myeloma, 25 reactions
- **D006-050R:** NXtec D006 Multiple Myeloma, 50 reactions
- **D006-100R:** NXtec D006 Multiple Myeloma, 100 reactions

NXtec D006-A1 Multiple Myeloma (hereafter: D006 Multiple Myeloma) is to be used in combination with:

1. NXtec Reagent Kit (Cat No: DRK01-IL, DRK05-IL, DRK20-IL)
2. Barcode plates:
NXtec Barcode Plate 1 (Cat No: BP01-IL (from lot 03-009-xxxxxx and higher))
NXtec Barcode Plate 2 (Cat No: BP02-IL (from lot 03-008-xxxxxx and higher))

N.B. The three-digit number between dashes (e.g. -008-) will increase with every new barcode plate lot.

3. Data analysis software Coffalyser digitalMLPA™ (Cat No: n.a.)

Volumes and ingredients

Volumes			Ingredients
D006-025R	D006-050R	D006-100R	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, Tris-HCl, EDTA, DTT

The 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 is available at www.mrcholland.com.

Precautions and warnings

For professional use only. Always consult the most recent product description AND the digitalMLPA NXtec 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

NXtec D006-A1 Multiple Myeloma is a **research use only (RUO)** assay for the detection of deletions, gains or amplifications of genes and chromosomal regions mentioned in Table 2 that are recurrently altered in multiple myeloma, such as 1p, 1q, 13q and 17p, as well as for the detection of *BRAF* p.V600E point mutation.

Multiple myeloma (MM) is a clonal B-cell disorder characterised by malignant proliferation of monoclonal plasma cells. MM cases present with a common histological and morphological diagnosis, however simultaneously displaying enormous genetic and molecular complexity as well as marked variations in clinical characteristics and patient survival. Recent progress in molecular cytogenetics has improved the understanding of pathogenesis of MM and also provided reasoning for molecular sub-classification of MM. Genetic alterations in MM are well characterised and include gross chromosomal rearrangements such as fusion genes, hyper-/hypodiploidy and also focal deletions. This probemix is designed to detect the majority of the primary and secondary CNAs in MM.

This probemix is not CE/FDA registered for use in diagnostic procedures. The digitalMLPA technique is covered by US patent 6,955,901 and corresponding patents outside the US and digitalMLPA products are sold under a license of Invitae corporation on patent US 9,624,533. The purchase of this product includes a license on these patents to use only this amount of product solely for the purchaser's own use.

Probemix content

A total number of 644 probes is included in D006-A1 Multiple Myeloma, this consists of:

- 189 target probes detecting copy number alterations involved in multiple myeloma (Table 2).
- One mutation-specific probe which will only generate probe reads when *BRAF* p.V600E point mutation is present (Table 2).
- 278 karyotyping probes, covering all existing chromosome arms (at the middle, near the centromeres and near the telomeres). 81 karyotyping probes are used as reference probes. See Table 3 for all chromosomal regions and genes included.
- More than 160 control probes and fragments are included: probes to aid in normalisation in case of copy number changes in tumour samples, probes for sample identification and probes for detection of errors or deviations when performing digitalMLPA assays, impurities in and fragmentation of the DNA samples, ligase and polymerase activity and extent of hybridisation.

The total number of probes can be used to calculate the number of reactions that can be combined into one sequencer run. See chapter "Amplicon Quantification by Illumina Sequencers" in the digitalMLPA NXtec Protocol or the calculator tool available at support.mrcholland.com.

Reference probes

The selected reference probes are a subset of karyotyping probes in regions that show minimal copy number changes in MM. This was determined using information from the Progenetix oncogenomic online resource, Database of Genomic Variants (DGV), Broad Institute TCGA Copy Number Portal, and Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census. As long as more than 50% of the sequences targeted by the reference probes have a normal copy number (CN=2) in the test sample, the correct baseline will be detected and data will be correctly normalised using Coffalyser digitalMLPA

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>

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

Tark – Transcript Archive: <http://tark.ensembl.org/>

digitalMLPA technique

digitalMLPA (Benard-Slagter et al. 2017) combines the robustness and simplicity of the trusted SALSA® MLPA® technology (Schouten et al. 2002) with next-generation sequencing. For NXtec products a specific protocol of the digitalMLPA technique is used. The principles of digitalMLPA and the protocol for NXtec products are described in the digitalMLPA NXtec Protocol (www.mrcholland.com).

digitalMLPA technique validation

Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using this NXtec probemix for the first time, or when pre-analytical steps, DNA extraction method or the instruments used are changed. This validation experiment should result in a standard deviation ≤ 0.10 for all reference probes with the exception of SNP- and mutation-specific probes.

Required specimens

Extracted DNA from bone marrow plasma cells, free from impurities known to affect digitalMLPA reactions. For more information see the digitalMLPA NXtec Protocol, section DNA sample treatment. The minimum percentage of tumour cells required for reliable analysis is 30% (Al Zaabi et al. 2010, Coll-Mulet et al. 2008). We would advise to use tumour samples with at least 50% tumour cell content to minimize the variation in tumour cell estimation, and to allow robust data-analysis and detection of potential subclonal aberrations. We therefore recommend to evaluate the tumour samples by a pathologist, and in case of low tumour cell percentage, to make use of enrichment methods, prior DNA extraction.

Reference samples

As X- and Y-chromosome specific probes are included in this probemix, at least three male reference samples AND three female reference samples need to be used per experiment. In instances where an experiment only contains patient samples of one gender, at least three reference samples of the same gender should be used. Pooled DNA from different genders can never be used as reference samples for D006 Multiple Myeloma analysis. 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. More information regarding the selection and use of reference samples can be found in the digitalMLPA NXtec 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 digitalMLPA experiments. In addition, reference standards from Horizon Discovery (<https://horizondiscovery.com/>) may also be used for BRAF p.V600E point-mutation detection. The quality of cell lines can change, therefore positive samples should be either acquired from quality assessed biological sample repositories or validated before use. Tables 1a and 1b contain a list of positive control samples that have been tested with D006 Multiple Myeloma at MRC Holland.

Table 1. Positive samples from biobanks tested by MRC Holland**Table 1a. Germline samples**

Coriell sample ID	Genomic aberration * ^ +
NA22977	1p36.33 heterozygous deletion
NA00803	1q23.1-q23.3 heterozygous deletion (including <i>FCRL5</i> , <i>SLAMF7</i> , <i>PBX1</i> -area, <i>PBX1</i>)
NA00214	1q31.3 heterozygous deletion
NA10401	Trisomy 2
NA11428	3p26.1-p26.2 heterozygous deletion (including <i>CRBN</i>) & 3q23-q29 heterozygous duplication
NA04127	3p24.1-p26.2 heterozygous duplication (including <i>CRBN</i>)
NA03563	3q23-q29 heterozygous duplication (including <i>ATR</i>)
NA10175	3q29 heterozygous duplication
NA10947	4p heterozygous duplication (including <i>FGFR3</i> , <i>NSD2</i>)
NA14489	4p16.3 heterozygous deletion (including <i>FGFR3</i> , <i>NSD2</i>) & 4p15.1-p15.32 heterozygous duplication (including <i>ATR</i>)
NA10313	4q33-q35.2 heterozygous duplication & 7q36.23 heterozygous deletion
NA00782	4q13.1-q31.21 heterozygous duplication
NA14131	5p15.2-p15.33 heterozygous deletion & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA14230	5q23.2-q31.1 heterozygous deletion
NA04371	5q35.3 heterozygous duplication
NA06801	6p25.2-p25.3 heterozygous duplication (including <i>IRF4</i>)
NA12721	6p22.3-p25.3 heterozygous duplication (including <i>JARID2</i> , <i>IRF4</i>) & 8p23.3 and <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA06802	6q25.3-q27 heterozygous deletion (including <i>PRKN</i>)
NA21698	6q27 heterozygous deletion
NA07081	7p heterozygous duplication (including <i>IKZF1</i>)
NA10925	7p12.2-p12.3 heterozygous deletion (including <i>IKZF1</i>)
NA05067	9p heterozygous duplication & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA10989	9p24.1-p24.3 heterozygous deletion & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA02819	9p22.3-p24.3 heterozygous duplication & 12q24.33 heterozygous deletion
NA13685	9q34.3 heterozygous duplication (including <i>TRAF2</i>) & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA03047	10p13-p14 heterozygous deletion
NA11672	10q11.22 heterozygous deletion
NA09596	11q21-q22.3 heterozygous deletion (including <i>BIRC2/3</i> , <i>ATM</i>) & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA15099	11q21-q25 heterozygous duplication (including <i>BIRC2/3</i> , <i>ATM</i> , <i>NCAPD3</i>)
NA07981	12p heterozygous triplication/homozygous duplication (including <i>LTBR</i> , <i>NCAPD2</i> , <i>CHD4</i> , <i>ETV6</i> , <i>CDKN1B</i> , <i>GPRC5D</i>)
NA07891	12q24.33 heterozygous duplication & 18q21.1-q23 heterozygous deletion
NA13721	13q14.11-q21.33 heterozygous deletion (including <i>RB1</i> , <i>DLEU1</i> , <i>DIS3</i>)
NA08254	13q34 heterozygous deletion
NA13410	14q32.31-q32.33 heterozygous duplication (including <i>TRAF3</i> , <i>IGHD/M</i>)
NA03184	Trisomy 15 (including <i>GABRB3</i>)
NA20375	15q12 heterozygous deletion (including <i>GABRB3</i>)
NA06226	16p13.12-p13.3 heterozygous duplication (including <i>TNFRSF17</i> (BCMA))
NA12074	16q22.1 heterozygous deletion
NA09687	16q23.1-q24.3 heterozygous duplication (including <i>WWOX</i>)
NA09209	17p13.3 heterozygous deletion
NA16445	17q25.3 heterozygous duplication
NA01359	Trisomy 18
NA02944	20p heterozygous duplication & 22q11.1-q11.21 heterozygous deletion
NA09868	21q22.2-q22.3 heterozygous deletion
NA07106	22q heterozygous duplication (including <i>SMARCB1</i>)
NA04626#	Trisomy X (including <i>KDM6A</i>)

Table 1b. Multiple myeloma cell line samples

DSMZ sample ID (name)	CNAs detected by D006 Multiple Myeloma probes * ^ +
ACC-163 (NCI-H929) #	gains: 1q21.1-q23.3 (including <i>ANP32E</i> , <i>MCL1</i> , <i>ADAR</i> , <i>CKS1B</i> , <i>FCRL5</i> , <i>SLAMF7</i> , <i>PBX1</i> -area, <i>PBX1</i>), 8p23.3-q24.21 (including <i>MYC</i>), 11q21-q25 (including <i>BIRC2/3</i> , <i>ATM</i> , <i>NCAPD3</i>), 18q21.1-q23, 19p13.13-p13.3, 20q (including <i>MAFB</i>) deletions: 1p21.2-p22.1 (including <i>EVI5</i> , <i>RPL5</i> , <i>CDC14A</i>), 1p12 (including <i>TENT5C</i>), 6q25.3-q27 (including <i>PRKN</i>), 7p22.1-p22.2, 10q11.21-q11.22, 12p11.22-p13.31 (including <i>LTBR</i> , <i>NCAPD2</i> , <i>CHD4</i> , <i>ETV6</i> , <i>CDKN1B</i> , <i>GPRC5D</i>), 13q (including <i>RB1</i> , <i>DLEU1/2/7</i> , <i>DIS3</i>), <i>IGHD/M</i> on 14q32.33 (homozygous), 19q13.42-q13.43, 20p, Xp (including <i>KDM6A</i>), Xq11.1-q22.1
ACC-430 (SK-MM-2)	gains: 8q, 11q13.3-q25 (including <i>CCND1</i> , <i>BIRC2/3</i> , <i>ATM</i> , <i>NCAPD3</i>), 18p, 18q21.1 deletions: 1p31.3-p32.3 (including <i>FAF1</i> , <i>CDKN2C</i> (homozygous), <i>DAB1</i>), 6q22.31-q27 (including <i>PRKN</i>), 8p, 9p22.3-p24.3, 13q12.3-q14.3 (including <i>RB1</i> , <i>DLEU1/2/7</i>), <i>IGHD/M</i> on 14q32.33, 16q (including <i>CYLD</i> , <i>WWOX</i>), 17p13.1-p13.3 (including <i>TP53</i>), 18q23, 22q11.21-q12.2 (including <i>SMARCB1</i>), chr. Y (homozygous)~
ACC-606 (KMS-12-PE) #	gains: 1q21.1-q23.3 (including <i>ANP32E</i> , <i>MCL1</i> , <i>ADAR</i> , <i>CKS1B</i> , <i>FCRL5</i> , <i>SLAMF7</i> , <i>PBX1</i> -area, <i>PBX1</i>), 1q44, 3q29, IRF4 at 6p25.3, 7p11.2-qter (including <i>IKZF1</i>), 8q24.21-q24.3 (including <i>MYC</i>), 9q21.12-q31.1, 10p13-p15.3, 11q13.3-q25 (including <i>CCND1</i> , <i>BIRC2/3</i> , <i>ATM</i> , <i>NCAPD3</i>), 13q34, 14q32.33 (excluding <i>IGHD/M</i>), 18p deletions: 1p32.3 (including <i>FAF1</i> , <i>CDKN2C</i> (homozygous)), 1p21.3-p22.1 (including <i>EVI5</i> , <i>RPL5</i>), 1p12-p13.1 (including <i>TENT5C</i>), 1q31.3, 4q31.21-q35.2, 5q, 9p22.3-p24.3, 10q11.21-q26.3, 11q11.2-q22.2, 13q12.3-q21.33 (including <i>RB1</i> , <i>DLEU1/2/7</i> , <i>DIS3</i>), 14q11.2-q22.2, <i>IGHM</i> on 14q32.33, 16q23.1-q24.3 (including <i>WWOX</i>), 17p (including <i>TP53</i>), 18q11.2, 18q21.1-q23, 19q, 20p12.3-p13, 22q11.1-q11.21, 22q12.2-q13.33, Xp (including <i>KDM6A</i> , homozygous for exon 4 probe), Xq11.1-q22.1

* Single probe findings are not shown in the tables above; only CNAs detected by two or more consecutive probes are listed.

+ Only gene names listed in Table 2 with two or more probes are indicated, however, the chromosomal bands with CNAs do contain probes (listed in Table 3).

^ Indicated CNA region is based on the chr. band (hg38) targeted by D006 Multiple Myeloma probes, however, the exact extent of CNA cannot be determined by this probemix.

Analysis of this female sample was done using >3 female reference samples.

~ Y chromosome CN status was determined based on the knowledge of the sample gender from public resources and checking the read counts for probes targeting the Y-chromosome in Coffalyser digitalMLPA "Ratios" excel output file. In this case, the median read count for Y-probes was 0, indicating a homozygous deletion.

Data analysis

Coffalyser digitalMLPA must be used for data analysis in combination with the appropriate lot-specific product sheet. For both, the latest version should be used. Coffalyser digitalMLPA is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. Normalisation of results should be performed within one experiment. The Coffalyser digitalMLPA User Manual contains technical guidelines and information on data evaluation/normalisation.

Interpretation of results

The expected results for (pseudo)autosomal probes are allele copy numbers of 2 (normal), 1 or 0 (deletion), ≥3 (gain). The same results can be expected for the X-chromosome-specific probes in female samples. For the X-chromosome-specific probes in male samples, expected copy numbers are 1 (normal), 0 (deletion) or 2 (gain).

The standard deviation of all probes in the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the inter ratio of the probes can be used to interpret digitalMLPA results when **reference samples of the same sex** have been used:

Copy number status	Inter ratio	
	autosomal probes / X-chromosome-specific probes in female samples	X- and Y-chromosome-specific probes in male samples
Normal	$0.85 \leq \text{ratio} \leq 1.15$	$0.60 \leq \text{ratio} \leq 1.40$
Deletion *	$\text{ratio} < 0.75$	$\text{ratio} < 0.40$
Gain ±	$\text{ratio} > 1.25$	$\text{ratio} > 1.60$
Ambiguous copy number	All other values	All other values

* Ratios might indicate a (subclonal) biallelic deletion when autosomal inter ratios are ≤0.30.

± Ratios might indicate an amplification when inter ratios are ≥2.15.

Please note that these above mentioned inter ratios are affected both by percentage of tumour cells and by possible subclonality. In case of a deletion that is subclonal and/or a lower percentage of tumour cells, the inter ratio may be higher than expected. For example, a monoallelic deletion in a sample with 50% tumour cell content or a monoallelic deletion present in 50% of the tumour cells, will result in an inter ratio around 0.75. However, the same (ambiguous) inter ratio of 0.75 will also be found in a sample with a biallelic deletion and a tumour cell percentage of 25%, or a subclone harbouring a biallelic deletion comprising 25% of all tumour cells. The digitalMLPA technique cannot discriminate between these two scenarios.

More information on this can be found on [our website](#).

General notes on digitalMLPA interpretation:

- Arranging probes according to chromosomal location facilitates interpretation of the results. Analysis of parental samples may be necessary for correct interpretation of complex results.
- 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 read count of several consecutive probes, in particular for probes located in or near a GC-rich region. The use of an alternative DNA extraction method or an additional purification step (e.g. with ethanol precipitation or silica column based kits) may resolve such cases. Control probes are present in all digitalMLPA probemixes that provide a warning for incomplete DNA denaturation. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can also lead to false-positive results.
- False positive results (gains): Contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe read count (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 digitalMLPA are pathogenic. 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. In some genes, intragenic deletions are known that result in very mild, or no disease (Schwartz et al. 2007). Duplications that include the first or last exon of a gene (e.g. exons 1-3) might in some cases 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.

D006-A1 Multiple Myeloma specific notes:

- The use of enrichment of CD138+ plasma cells is highly recommended as it 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. 2015).
- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood-derived germline samples, which could result in warnings in Coffalyser digitalMLPA about reference probe quality.
- Due to high numbers of CNAs in MM samples, the control probes included for sample identification will not be able to indicate sample DNA contamination, but can still be used for reliable sample identification (SNP code in Coffalyser digitalMLPA reports).

Limitations of the procedure

- Translocations involving the IgH locus are the most common primary genetic events in MM, however digitalMLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect most copy number neutral inversions or translocations. Even when digitalMLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.

- In majority of MM samples, the main cause of genetic defects in the *ATM*, *DIS3*, *TENT5C* (*FAM46C*) genes covered by D006 Multiple Myeloma are small (point) mutations, which will not be detected by using this probemix.
- digitalMLPA cannot distinguish haploid or complete triploid or tetraploid samples (with a loss of gain of all chromosomes) as compared to diploid samples, as the Coffalyser software determines a baseline based on the reference probes in each individual sample.
- **Warning:** Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results. Sequence changes can reduce the probe read count by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
- digitalMLPA analysis on tumour samples provides information on the average situation in the cells from which the DNA sample was purified. In addition, subclonality of the aberration affects the inter ratio of the corresponding probe. Furthermore, there is always a possibility that reference probes *do* show a copy number alteration especially in samples with complex karyotypes or ploidy changes potentially complicating data normalisation (as digitalMLPA is a relative technique). In such cases knowledge from DNA indexing or karyotyping can support accurate interpretation of probe inter ratios.

Confirmation of results

Copy number changes of multiple consecutive probes detected with D006-A1 Multiple Myeloma should be verified by another method when possible. MLPA probemixes are available for several genes and chromosomal regions in D006-A1 Multiple Myeloma. Most of these MLPA probemixes contain probes with a different ligation site that can be used for initial confirmation of results (see section 'Related SALSA® MLPA® probemixes' in this product description). Alternatively, copy number changes can be confirmed by another independent technique such as long range PCR, qPCR, array CGH, FISH or Southern blotting.

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 result was obtained.

COSMIC mutation database

<http://cancer.sanger.ac.uk/cosmic>. We strongly encourage users to deposit positive results in the COSMIC. 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 to MRC Holland: info@mrcholland.com.

Table 2. D006-A1 Multiple Myeloma probe targets

Chromosomal position (hg38)		Gene	NM sequence (a)	# probes / # exons in gene	Gene / region length	Remarks
1p (33 probes)	1p36.33	<i>TMEM240</i>		1 probe per gene	43 Mb	
		<i>CFAP74</i>				
	1p35.1	<i>YARS1</i>				
	1p34.1	<i>HECTD3</i>				
	1p32.3	<i>FAF1</i>	NM_007051.3	2/19	523 kb	
		<i>CDKN2C</i>	NM_078626.3	3/2	4.9 kb	
		<i>ORC1</i>		1 probe per gene	4.2 Mb	
	1p32.2	<i>PLPP3</i>				

Chromosomal position (hg38)		Gene	NM sequence ^(a)	# probes / # exons in gene	Gene / region length	Remarks
		DAB1	NM_001365792.1	4/15	429 kb	
	1p31.3	LEPR		1 probe		
	1p22.1	EVI5	NM_001350197.2	4/20	276 kb	
		RPL5	NM_000969.5	3/8	9.9 kb	
	1p21.3	DPYD		1 probe per gene	3.0 Mb	
	1p21.2	DBT				
		CDC14A	NM_003672.4	4/16	168 kb	
	1p13.1	SLC22A15		1 probe		
	1p12	TENT5C	NM_017709.4	2/2	22.3 kb	previous name FAM46C
SPAG17			1 probe			
1q (29 probes)	1q21.1	PDZK1		1 probe per gene	1.9 Mb	
	1q21.2	BCL9				
		ANP32E	NM_030920.5	2/7	17.7 kb	
		RPRD2		1 probe		
		MCL1	NM_021960.5	2/3	5.1 kb	
	1q21.3	NUP210L		1 probe		
		ADAR	NM_001111.5	2/15	26.1 kb	
		CKS1B	NM_001826.3	3/3	4.6 kb	
	1q23.1	FCRL5	NM_031281.3	3/17	39.1 kb	
	1q23.3	SLAMF7	NM_021181.5	2/7	15.6 kb	
		NUF2		1 probe	81.3 Mb	gene-poor region; probes are targeting 113-238 kb upstream of PBX1
		PBX1-area		5 probes		
			PBX1			
	1q31.3		KCNT2			
		DENND1B				
		1q44	ADSS2			
	DESI2					
	3p26.2	CRBN	NM_016302.4	3/11	29.7 kb	
	3q23	ATR	NM_001184.4	3/47	129.5 kb	
4p16.3	FGFR3	NM_000142.5	5/18	15.6 kb		
	NSD2	NM_001042424.3	2/22	111 kb	previous name WHSC1	
6p25.3	IRF4	NM_002460.4	2/9	19.7 kb		
6p22.3	JARID2	NM_004973.4	2/18	276.0 kb		
6q26	PRKN	NM_004562.3	2/12	1.4 Mb	previous name PARK2	
7p12.2	IKZF1	NM_006060.6	3/8	100.4 kb		
7q34	BRAF⁺	NM_004333.6	1 mutation-specific probe	205.6 kb	p.V600E⁺ (c.1799T>A)	
8q24.21	MYC	NM_002467.6	3/3	6.7 kb		
9q34.3	TRAF2	NM_021138.4	2/11	40.1 kb		
11q13.3	CCND1	NM_053056.3	3/5	13.3 kb		
11q22.2	BIRC3		1 probe	31.5 kb		
	BIRC2	NM_001166.5	2/9	21.9 kb		
11q22.3	ATM	NM_000051.4	4/63	146.0 kb		

Chromosomal position (hg38)		Gene	NM sequence ^(a)	# probes / # exons in gene	Gene / region length	Remarks	
11q25		NCAPD3	NM_015261.3	2/35	73.9 kb		
12p13.31		LTBR	NM_002342.3	2/10	7.4 kb		
		NCAPD2	NM_014865.4	2/32	37.9 kb		
		CHD4	NM_001273.5	2/40	37.3 kb		
12p13.2		ETV6	NM_001987.5	3/8	245.7 kb		
12p13.1		CDKN1B	NM_004064.5	2/3	5.0 kb		
		GPRC5D	NM_018654.2	3/4	11.6 kb		
13q (23 probes)	13q12.3	KATNAL1		1 probe per gene	13.2 Mb		
	13q14.11	ENOX1					
	13q14.2-q14.3	RB1	NM_000321.3	5/27	178.1 kb	21.2 Mb	
		RCBTB2		1 probe per gene			
		DLEU2					
		KCNRG					
		MIR15A					
		DLEU1	NR_109973.1	2/7	1 probe per gene		
		DLEU7		1 probe per gene			
		RNASEH2B					
		ATP7B					
		VPS36					
		PCDH8					
	13q21.33	KLHL1	NM_014953.5	2/21	29.7 kb		
	DIS3						
	13q34	ARHGEF7		1 probe per gene	2.5 Mb		
		GRK1					
14q32.32		TRAF3	NM_145725.3	2/12	134.0 kb		
14q32.33		IGHD	NG_001019.6	2/7	8.9 kb		
		IGHM		4/6	4.5 kb		
15q12		GABRB3	NM_000814.6	2/9	229.5 kb		
16p13.13		TNFRSF17	NM_001192.3	4/3	2.9 kb	BCMA in literature	
16q12.1		CYLD	NM_001378743.1	2/19	59.9 kb		
16q23.1		WWOX	NM_016373.4	2/9	1.1 Mb		
17p (20 probes)	17p13.3	VPS53		1 probe per gene	1.7 Mb		
		NXN					
	17p13.1	TP53	NM_000546.6	14/11	19.1 kb	9.0 Mb	
		PIK3R6		1 probe per gene			
		USP43					
	17p11.2	RAI1			MIR33B		
17q12-q21.1		IKZF3	NM_012481.5	2/8	106.6 kb		
17q21.31		MAP3K14	NM_003954.5	2/16	53.9 kb		
20q12		MAFB	NM_005461.5	2/1	3.4 kb		
22q11.23		SMARCB1	NM_003073.5	5/9	51.0 kb		
Xp11.3		KDM6A	NM_001291415.2	2/30	239.6 kb		

Genes covered by two or more probes are indicated in **bold**.

(a) NM sequence and MANE exon numbering: The exon numbering and NM_ sequence used are based on MANE project (release version 1.0) retrieved on 10/2022. As changes to the MANE database can occur after release of this product description, exon numbering may not be up-to-date. Exon numbering used here may differ from literature. When a MANE transcript is not available, then the exon numbering is based on the NM, NR or NG sequence.

+ Please note that due to high nucleotide sequence similarity of mutated V600E (GTG to GAG single nucleotide variation) and V600K (GTG to AAG double nucleotide variation) codons, the *BRAF* V600E probe included in this probemix might result in small number of reads on a sample with V600K mutation.

More information on the location, mutation details and warnings of the probes present in this probemix can be found in the Probe Information File (PIF) available [on the product page at www.mrcholland.com](http://www.mrcholland.com).

Table 3. Karyotyping probes

2p25.3	TMEM18	5q31.3	PCDHAC1
2p25.3	COLEC11 §	5q31.3	PCDHAC2
2p22.3	SPAST §	5q31.3	PCDHB2
2p22.2	VIT §	5q31.3	PCDHB10
2p15	PEX13 §	5q31.3	SLC25A2
2p11.2	REEP1	5q31.3	TAF7
2q11.2	CNNM3 §	5q31.3	PCDHGA11
2q11.2	NPAS2 §	5q32	SH3TC2
2q24.3	SCN1A §	5q35.3	COL23A1
2q31.1	ABCB11 §	5q35.3	MAPK9
2q32.2	COL3A1 §	6p25.2	GMDS-DT §
2q37.3	CAPN10	6p25.2	SERPINB6
2q37.3	KIF1A	6p25.2	PXDC1 §
3p26.2	TRNT1	6p25.2	ECI2 §
3p26.1	SUMF1	6p22.3	KIAA0319
3p24.2	NR1D2	6p22.1	ZFP57 §
3p24.1	NEK10	6p21.33	TNF §
3p12.3	CNTN3	6p12.3	PKHD1
3p11.1	HTR1F	6p12.1	RAB23 §
3q11.2	CPOX	6p11.2	PRIM2 §
3q23	CLSTN2	6q12	EYS
3q24	SLC9A9	6q13	COL19A1
3q29	OPA1	6q13	RIMS1
3q29	ACAP2	6q22.31	TBC1D32
3q29	RUBCN	6q22.33	LAMA2
4p16.3	LETM1 §	6q23.3	TNFAIP3
4p16.3	ADD1 §	6q25.3	TFB1M
4p15.32	LDB2	6q25.3	WTAP
4p15.31	KCNIP4 §	6q25.3	IGF2R
4p15.2	SEL1L3	6q27	SMOC2
4p15.1	PCDH7 §	6q27	ERMARD
4p13	ATP8A1 §	7p22.2	SDK1
4q13.1	TECRL §	7p22.1	RADIL
4q13.3	UGT2A1	7p15.3	RAPGEF5
4q21.1	G3BP2 §	7p15.3	STK31
4q25	HADH §	7p12.3	ADCY1
4q31.21	INPP4B §	7p12.3	ABCA13
4q31.21	ZNF827 §	7p11.2	LANCL2
4q31.23	NR3C2 §	7q11.21	KCTD7
4q33	CLCN3 §	7q11.22	GALNT17
4q35.1	CYP4V2	7q31.1	PNPLA8
4q35.2	TRIML1	7q31.1	IFRD1
5p15.33	IRX4	7q36.3	RBM33
5p15.31	NSUN2	7q36.3	DYNC211
5p15.2	CTNND2	8p23.3	FBXO25
5p15.2	DNAH5	8p23.3	CLN8
5p13.3	NPR3	8p23.1	GATA4
5p13.2	TTC23L	8p21.3	GFRA2
5q11.2	IL31RA	8p21.3	TNFRSF10B
5q11.2	MIER3	8p21.3	TNFRSF10A
5q13.3	CERT1	8p21.2	NEFL
5q14.3	ADGRV1	8p12	RBPMS
5q23.2	SNCAIP	8p12	GSR
5q23.2	MARCHF3	8p11.23	ZNF703
5q31.1	SLC22A5	8q11.21	SNTG1 §
5q31.2	MYOT	8q12.2	CHD7 § +
5q31.2	GFRA3	8q21.3	RMDN1 §
5q31.2	CTNNA1	8q21.3	CPNE3 §
5q31.3	PCDHA1	8q22.3	RRM2B

Chromosomal position (hg38)	Gene
8q24.3	SLC39A4 §
9p24.3	DOCK8
9p24.1	JAK2
9p24.1	GLDC
9p22.3	FREM1
9p22.3	BNC2
9p13.2	FBXO10
9p13.2	DCAF10
9q21.12	TRPM3
9q31.1	ALDOB
9q34.3	COL5A1
9q34.3	GRIN1
9q34.3	EHMT1
10p15.3	ZMYND11 §
10p15.3	DIP2C §
10p15.2	PFKP
10p15.2	PITRM1
10p14	ECHDC3 §
10p13	NMT2 §
10p13	ITGA8
10p11.21	CUL2 §
10p11.21	ZNF25 §
10q11.21	MARCHF8 §
10q11.22	ARHGAP22 §
10q22.2	KAT6B §
10q25.2	ADD3 §
10q26.3	INPP5A §
10q26.3	KNDC1
11p15.5	RIC8A
11p15.5	DEAF1
11p14.3	ANO5 +
11q12.3	BEST1
11q21	MTMR2
11q25	NTM
11q25	JAM3
12p13.33	WNK1
12p13.33	CACNA2D4
12p13.33	TSPAN9
12p13.31	CD27
12p13.31	VAMP1
12p13.2	BCL2L14
12p13.2	LRP6
12p13.2	BORCS5
12p12.3	AEBP2
12p11.22	REP15
12p11.22	MANSC4
12p11.22	PTHLH
12p11.22	TMTC1
12q12	KIF21A §
12q12	NELL2 §
12q15	MDM1 §
12q21.2	E2F7 §
12q23.1	NEDD1 §
12q23.1	SLC17A8 §
12q24.22	NOS1 §
12q24.33	GALNT9
12q24.33	PGAM5
14q11.2	CHD8 §
14q22.1	DDHD1 §
14q22.2	SAMD4A §

Chromosomal position (hg38)	Gene
14q24.3	NPC2 §
14q32.31	DYNC1H1 §
14q32.33	COA8
14q32.33	MTA1
14q32.33	TEDC1
15q21.1	SPG11
15q21.1	FBN1
15q22.2	VPS13C
15q22.31	USP3
15q23	CLN6
15q26.3	IGF1R
15q26.3	CHSY1
15q26.3	TM2D3
16p13.3	DECR2 §
16p13.3	IFT140 §
16p13.13	TXNDC11 §
16p13.12	CPPED1
16p11.2	HIRIP3
16p11.2	ITGAL §
16q11.2	GPT2
16q12.1	LONP2
16q13	SLC12A3
16q22.1	SLC12A4
16q22.1	DUS2
16q23.2	MAF
16q24.3	ANKRD11
16q24.3	GAS8
17q11.2	PSMD11
17q23.2	MED13
17q25.3	CCDC57
17q25.3	CSNK1D
18p11.31	LPIN2 §
18p11.31	TGIF1 §
18p11.21	GNAL §
18p11.21	SPIRE1 §
18p11.21	RNMT §
18q11.2	NPC1 § +
18q21.1	LOXHD1 §
18q21.1	LIPG §
18q23	CTDP1 §
18q23	TXNL4A
19p13.3	PLPP2
19p13.2	TEX45
19p13.13	GET3
19p13.13	GCDH
19p13.13	STX10
19p13.11	GMIP
19q13.11	SLC7A9
19q13.2	ACP7
19q13.2	COQ8B
19q13.42	DNAAF3
19q13.43	SLC27A5
20p13	RBCK1
20p13	RSPO4
20p13	TGM6 §
20p12.3	TRMT6 §
20p12.2	PLCB4 §
20p11.23	RIN2 §
20p11.21	APMAP §
20q11.22	ACSS2 §

Chromosomal position (hg38)	Gene
20q11.22	<i>EDEM2</i> §
20q11.23	<i>SAMHD1</i> §
20q13.12	<i>SLC13A3</i> §
20q13.13	<i>STAU1</i> §
20q13.33	<i>OSBPL2</i>
20q13.33	<i>UCKL1</i>
21q11.2	<i>RBM11</i>
21q11.2	<i>HSPA13</i>
21q22.11	<i>ITSN1</i>
21q22.2	<i>PSMG1</i>
21q22.3	<i>PDE9A</i>
21q22.3	<i>PWP2</i>
21q22.3	<i>TSPEAR</i>
22q11.1	<i>GAB4</i>
22q11.21	<i>CECR2</i>
22q11.21	<i>HIRA</i>
22q12.2	<i>NF2</i>
22q12.2	<i>ZMAT5</i>

Chromosomal position (hg38)	Gene
22q12.2	<i>SFI1</i>
22q12.3	<i>LARGE1</i>
22q13.2	<i>EP300</i>
22q13.31	<i>TRMU</i>
22q13.33	<i>BRD1</i>
Xp22.31	<i>ANOS1</i>
Xp22.11	<i>ACOT9</i>
Xp11.22	<i>FGD1</i>
Xq11.1	<i>ARHGEF9</i>
Xq13.3	<i>ZDHHC15</i>
Xq22.1	<i>NXF3</i>
Xq28	<i>CLIC2</i>
Xq28	<i>TMLHE</i>
Yp11.2	<i>PCDH11Y</i>
Yp11.2	<i>TBL1Y +</i>
Yq11.221	<i>USP9Y</i>
Yq11.223	<i>KDM5D</i>
Yq11.223	<i>RPS4Y2</i>

Odd numbered chromosomes are highlighted grey.

§ Used as reference probe for normalisation purposes in data analysis.

+ Two probes are included for this gene.

Note: No karyotyping probes are present in the acrocentric chromosome p-arms.

More information on the location, mutation details and warnings of the probes present in this probemix can be found in the [Probe Information File \(PIF\)](#) available at www.mrcholland.com.

Table 4. Related SALSA® MLPA® probemixes

Related probemix	Coverage ±	Can be used for confirmation ^
P425 Multiple Myeloma	Contains probes for 1p, 1q, 5q31, chr. 9, 12p13, 13q14-q22, <i>TRAF3</i> , chr. 15, <i>CYLD</i> , <i>WWOX</i> , <i>TP53</i> .	no
P056 TP53	Contains probes for each exon of <i>TP53</i> .	no
P047 RB1	Contains probes for each exon of <i>RB1</i> , except exon 15.	yes *
P041/P042 ATM-1/-2	Contain probes for each exon of <i>ATM</i> .	yes *
P088 Oligodendroglioma 1p-19q	Contains probes for chromosomal arms 1p, 1q, 19p, 19q.	yes *
P380 Wilms' tumour	Contains probes for 1p, 1q, 16p, 16q, <i>TP53</i> .	yes *
P480 WHS & Achondroplasia	Contains probes for 4p16.3 including <i>NSD2</i> and <i>FGFR3</i> .	yes *
P244 AIP-MEN1-CDKN1B	Contains six probes on chromosomal arm 12p (including <i>CDKN1B</i>).	yes *
P202 IKZF1-ERG	Contains probes for each exon of <i>IKZF1</i> and 14q32.33, among others.	yes *
P335 ALL- <i>IKZF1</i>	Contains probes for <i>IKZF1</i> , <i>ETV6</i> and <i>RB1</i> , among others.	yes *
P037/P038/P040 CLL-1/-2	Contain probes for 2p, 6q, 8p, 8q (<i>MYC</i>), 11q (<i>ATM</i>), chr. 12, 13q14 (<i>RB1</i> , <i>DLEU1/2</i>), <i>TP53</i> , 14q32.33, 17p (<i>TP53</i>), chr. 19.	yes *
P258 SMARCB1	Contains probes for each exon of <i>SMARCB1</i> and flanking regions on 22q.	yes *
P414 MDS	Contains probes for chr. 3, 5q, 7q, 8q, 11q, 12p (<i>ETV6</i>), chr. 17 (<i>TP53</i>), chr. 19, 20q and Y chromosome.	yes *
P323 CDK4-HMGA-MDM2	Contains probes on chromosomal arms 12p and 12q.	yes *
P105 Glioma-2	Contains nine probes for <i>TP53</i> , among others.	no
P051/P052 Parkinson mix 1/2	Contain probes for each exon of <i>PRKN</i> .	yes *
P064 Microdeletion Syndromes-1B	Contains nine probes for <i>NSD2</i> , among others.	yes *
P377 Hematologic Malignancies	Contains several probes for <i>IKZF1</i> , <i>MYC</i> , <i>ATM</i> , <i>ETV6</i> , 13q14, <i>TP53</i> , among others.	yes *
P078 Breast tumour	Contains probes for <i>MYC</i> , <i>CCND1</i> , among others.	yes
P451 Chromosome 16	Contains probes on chromosomal arms 16p and 16q.	yes *
P343 Autism 1	Contains 26 probes on chromosomal arm 15q.	yes *
P298 BRAF-HRAS-KRAS-NRAS	Contains a mutation-specific probe for <i>BRAF</i> p.V600E, among others.	yes
P301/P302/P303 Medulloblastoma mix 1/2/3	Contain probes for chr. 1, 2, 3, 4q, 5q, 6, 7, 8, 9, 10, 14q, 16, 17, 20.	yes *

± Only genes or chromosomal regions included in D006 Multiple Myeloma are mentioned in this table. SALSA® MLPA® probemixes additionally contain probes for genes not mentioned in this table.

^ Probemixes can be used for confirmation when ligation sites are different between D006 Multiple Myeloma probes and the probes in the corresponding probemixes. Of note, this statement concerns the majority of the probes in a probemix and does not mean that all probes always have a different ligation site. For more information, please contact info@mrcholland.com.

* The reference probes included in this SALSA® MLPA® probemix have not been optimised for MM samples. If the sample derived from MM harbours multiple copy number alterations on the genomic locations of the reference probes, the normalisation can be compromised and reliable result interpretation will not be possible with this probemix.

References

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- 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 D006 Multiple Myeloma


- Croft J et al. (2021). Copy number evolution and its relationship with patient outcome—an analysis of 178 matched presentation-relapse tumor pairs from the Myeloma XI trial. *Leukemia*. 35:2043-53.
- Kosztolanyi 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.
- Menezes K et al. (2020). High-Throughput Molecular Cancer Cell Line Characterization Using Digital Multiplex Ligation-Dependent Probe Amplification for Improved Standardization of in Vitro Research. *J Mol Diagn*. 22:1179-88.

D006 Multiple Myeloma product history	
Version	Modification
A1	First release.

Implemented changes in the product description
<p>Version A1-05 – 18 July 2025 (05)</p> <ul style="list-style-type: none"> - Products' names were updated throughout the document from SALSA® digitalMLPA™ to NXtec. - Modifications in the 'Required specimens' section regarding DNA extraction and tumour cell percentage. - Additional information provided in 'Limitations of the procedure' section regarding samples with triploid, tetraploid or complex karyotypes. - Performance characteristics section is removed. - 'Interpretation of results' section in the table the borders for normal samples were updated: < was replaced for ≤. - Product description adapted to a new template. <p>Version A1-04 – 24 January 2025 (03)</p> <ul style="list-style-type: none"> - Added inter ratio values for interpretation of X- and Y-chromosome-specific probe results in 'Interpretation of results' section on page 5. - Minor textual corrections. <p>Version A1-03 – 15 November 2024 (03)</p> <ul style="list-style-type: none"> - Specified the total number of probes and added a paragraph regarding the calculation of the number of reactions that can be combined into one sequencer run in 'Probemix content' section on page 2. - "Interpretation of results" section completely rewritten: new cut off values for deletions and gains introduced on page 5. - Minor textual changes. <p>Version A1-02 – 08 January 2024 (03)</p> <ul style="list-style-type: none"> - Replaced "SALSA digitalMLPA" with "SALSA® digitalMLPA™" where applicable.

- To be used with: section restructured and reagent kits (Cat No: DRK05-IL, DRK20-IL) added.
 - Barcode plate names and lot numbers updated.
 - Added sections: Ingredients, SDS note, Storage and handling, information on shelf life and safe disposal.
 - Added note on page 9 about *BRAF* V600E mutation-specific probe giving small read count also on samples with V600K mutation.
 - Small rearrangement of CNA region order for ACC-606 cell line in Table 1b on page 4.
 - Updated links to our website to <https://www.mrcholland.com> throughout the document.
 - Various minor textual changes.
- Version A1-01 – 08 November 2022 (03)
- Not applicable, new document.

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

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