Product Description
NXtec™ D008-A1 DMD

To be used with the digitalMLPA NXtec Protocol.

Version A1
Check the version of your product on the probemix label to ensure you are reading the appropriate product description. For complete product history see page 7.

Catalogue numbers
- **D008-025R:** NXtec D008 DMD, 25 reactions
- **D008-050R:** NXtec D008 DMD, 50 reactions
- **D008-100R:** NXtec D008 DMD, 100 reactions

NXtec D008-A1 DMD (hereafter: D008 DMD) 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 03-012-xxxxxx and higher))
   NXtec Barcode Plate 3 (Cat No: BP03-IL (from lot 03-010-xxxxxx and higher))
   NXtec Barcode Plate 4 (Cat No: BP04-IL (from lot 03-011-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™ 2.6.0 or higher.

Volumes and ingredients

Volumes			Ingredients
D008-025R	**D008-050R**	**D008-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, Tris-HCl, EDTA, DTT

The probemix is not known to contain any harmful agents. 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). Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard.

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened upon arrival. 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 D008-A1 DMD is a **research use only (RUO)** assay for the detection of deletions or duplications in the dystrophin (*DMD*) gene, associated with Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). This assay includes additional coverage upstream and downstream of *DMD* to aid determination of the extent of *DMD* deletions or duplications, as well as detection of X chromosome aneuploidies.

Germline defects in the *DMD* gene are the most common cause of Duchenne and Becker muscular dystrophies. Both of these X-linked recessive disorders occur predominantly in males. Female carriers may occasionally show symptoms due to skewed X-inactivation, leading to reduced dystrophin expression and a variable clinical phenotype (Korotkova et al. 2025; Sun et al. 2024; Yoshioka et al. 1998).

Variants in the *DMD* gene associated with DMD/BMD most commonly include deletions and duplications affecting one or more complete exons (Duan et al. 2021). Such copy number variations (CNVs) may be missed by standard sequence analysis, whereas most can be detected using the digitalMLPA technique.

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 Labcorp 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 of 403 probes are included in D008-A1 DMD:
- 158 probes detecting copy number variations in the *DMD* gene. Two probes are included for each exon of the gene. See the Probe Information File (PIF) and Table 1 for more details.
- 50 probes distributed across the X chromosome.
- 53 reference probes distributed across nearly all autosomal chromosomes.
- More than 120 control probes and fragments are included to determine quality of the experiment, the reaction, and the sample DNA.

The total number of probes mentioned above 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
A selection of 53 probes, distributed across nearly all autosomal chromosomes, is designated as reference probes.

Useful resources for 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. NXtec is a product line within the digitalMLPA technology. 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.

Required specimens and sample treatment
Extracted DNA, free from impurities known to affect digitalMLPA 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)

Ideally, 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, section DNA sample treatment.

Positive control 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. MRC Holland cannot provide positive DNA samples. As cell line quality may change over time, the actual copy number variation in a sample may differ from that indicated by the manufacturer. A list of positive control samples that have been tested with D008 DMD at MRC Holland can be found on the assay-specific product page.

Data analysis and reference samples
For data analysis, use the latest version of MRC Holland's free data analysis software Coffalyser digitalMLPA, (mrcholland.com) in combination with the latest relevant lot-specific product sheet. 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.

Two approaches can be used to analyse D008-A1 DMD data, depending on the experimental design and sample characteristics. Analysis with dedicated reference samples is strongly preferred, since D008-A1 DMD targets a single gene and there is a high likelihood of multiple samples harbouring the same aberration, thus affecting normalisation and result interpretation. Analysis without dedicated reference samples can be used on large heterogenous sample sets, as outlined below.

Analysis with dedicated reference samples
If the test sample set is small, includes multiple samples from the same family, and/or includes individuals with (suspected) DMD/BMD, inclusion of appropriate reference samples in the experiment is essential.

These reference samples **should show no aberrations across any** targeted regions. A sufficient number (≥3) of reference samples from unrelated individuals should be included in each digitalMLPA experiment for data normalisation. If the experiment includes more than 21 test samples, add one reference sample per additional seven test samples. Selected reference samples should be defined as "reference" and test samples as "test" in the Coffalyser definition file.

Analysis without dedicated reference samples
When large, heterogenous DNA sample sets from unrelated individuals are tested with D008-A1 DMD, it is unlikely that a substantial number of the samples will have the same copy number change. In this case, using dedicated reference samples **is not necessary**, and all samples can be used for inter-normalisation (for more details on data normalisation, see the Coffalyser digitalMLPA User Manual). When defining this experiment in Coffalyser digitalMLPA, all samples should be set to sample type "Test". The minimum number of required samples needs to be determined experimentally (read the background on our Support Portal).

Interpretation of results

Sample Report PDF files always show all X chromosome probe results, regardless of whether aberrations or quality warnings are triggered. Consult the Excel Report for a full overview of results including the control probe results.

The expected results for autosomal probes and X chromosome probes in female samples are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous duplication) or ≥4 (amplification). For X chromosome probes in male samples, expected copy numbers are 1 (normal), 0 (deletion) or 2 (duplication). Please note that there are cases in which higher number of X chromosomes are found in either female or male samples.

The standard deviation of all reference 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.

Copy number status **Autosomal sequences / X chromosome sequences in females (XX)** &	**Results**
Normal	0.80 ≤ ratio ≤ 1.20
Homozygous deletion	ratio = 0 ±
Heterozygous deletion / Turner syndrome (45,X)	0.40 < ratio < 0.65
Heterozygous duplication/gain	1.30 < ratio < 1.65
Heterozygous triplication/ Homozygous duplication/gain	1.75 < ratio < 2.15
Ambiguous copy number	All other values
X chromosome sequences in males (XY) &	**Results**
Normal	0.60 ≤ ratio ≤ 1.40
Hemizygous deletion	ratio = 0 ±
Duplication / Klinefelter syndrome (47,XXY)	1.60 < ratio < 2.25
Ambiguous copy number	All other values

& For inter-normalisation of X chromosome probes, the sex of the samples in the reference population in the experiment is automatically accounted for. Therefore, a copy number of 2 in female samples and a copy number of 1 in male samples both give a result of ~1.00, regardless of the sex of the samples in the reference sample population.
± Background signal: Beware that some probes may have minimal background signal, and therefore results close to, but not exactly 0.

D008-A1 DMD can also detect X chromosome aneuploidies, which can aid the interpretation of *DMD* results. An X chromosome aneuploidy can be interpreted using the 50 non-DMD probes distributed over the X chromosome. The table below illustrates the relationship between autosomal and X chromosome probes and the corresponding chromosomal aneuploidy. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a single nucleotide variant (SNV) influencing a single probe. It is important to use Coffalyser digitalMLPA to determine the significance of values found.

Sample			**Results** §	
Karyotype ø	**Sample type**	**Sex recognition by Coffalyser digitalMLPA**	Autosomal probes	X chromosome probes
46,XY	Male	Male	~1.00	~1.00
46,XX	Female	Female	~1.00	~1.00
45,X #	Turner syndrome	Female	~1.00	~0.50
47,XXX	Triple X syndrome	Female	~1.00	~1.50
47,XXY #	Klinefelter syndrome	Male	~1.00	~2.00

§ Please note that these calculations are theoretical.
ø D008-A1 DMD cannot accurately determine triploid genotypes (69,XXX; 69,XXY; 69,XYY) or higher-order polyploidy.

As XXY individuals are identified as males by Coffalyser digitalMLPA, the inter-normalisation is performed using male reference samples. As X0 individuals are identified as females by Coffalyser digitalMLPA, the inter-normalisation is performed using female reference samples. The sex of the samples in the reference population in the experiment is automatically accounted for, regardless of the actual sex of the reference samples included.

General notes on digitalMLPA interpretation:

- 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) in the target sequence detected by a probe can also lead to false-positive results.
- False positive duplication results: 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. 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 or flanking probes are unlikely to have any relation to the condition analysed.
- In the Excel report, a set of control probes are reported (identified by the probe function = CTRL) that can help troubleshoot the experiment in case of errors or warnings shown in the PDF reports or the sample quality tab of the Excel report. The Coffalyser digitalMLPA software checks these control probes against criteria set by MRC Holland. Only data that meet the quality requirements are suitable for result interpretation. See the Coffalyser digitalMLPA User Manual for more information on analysis, and the Coffalyser digitalMLPA General Protocol for details on troubleshooting.

D008-A1 DMD specific note:

- The use of cut-off values to distinguish between normal copy number states and duplications/trisomies or deletions/monosomies may limit the detection of mosaicism. Samples in which multiple DMD or X chromosome probes show ambiguous results should be interpreted with caution, and confirmatory testing using an alternative method is recommended.

Limitations of the procedure

- While, in most populations, the majority of pathogenic variants in the *DMD* gene are large deletions or duplications, smaller sequence variants (e.g. SNVs and small indels) account for a minority of cases and are not detected by D008-A1 DMD.
- 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.
- **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.

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 result was obtained.

D008-A1 DMD contains two target probes per *DMD* exon. This increases the robustness of this digitalMLPA assay against SNVs that could cause a decrease in a single target probe, leading to a false positive result. We recommend verifying copy number changes of multiple consecutive probes with another independent technique where possible, such as long range PCR, qPCR, array CGH, FISH or Southern blotting.

Please report false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

ClinVar database

We strongly encourage users to deposit positive results in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Table 1. D008-A1 DMD probe targets

Gene	Chr. band (hg38)	NM sequence (MANE Select) ◊	probes / exons	Gene length (kb)	Disorder
DMD	Xp21.2 – Xp21.1	NM_004006.3	158/79 (2 probes per exon)	2092.3	**Duchenne & Becker Muscular Dystrophy** OMIM: https://www.omim.org/entry/310200 https://www.omim.org/entry/300376 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1119/
	• Duplication of the complete *DMD* gene is not associated with disease. 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. One should be cautious with the prediction of an expected phenotype based on genotype. Factors like in-frame/out of-frame, extent and location of mutations in the *DMD* gene have different influences on the phenotype. The http://www.dmd.nl/ website has a tool to predict the effect of the deletions/duplications of exons on the reading frame.				

◊ The exon numbering and NM_ sequence used have been retrieved on 04/2026. 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.

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.

References

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- Yoshioka M et al. (1998). Skewed X inactivation in manifesting carriers of Duchenne muscular dystrophy. Clinical genetics. 53:102-107.

D008 DMD product history	
Version	*Modification*
A1	First release

Implemented changes in the product description
Version A1-01 – 26 May 2026 (05) - Not applicable, new document.

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