Product Description
NXtec™ D024-A1 KaryoProfiler

To be used with the digitalMLPA NXtec Protocol.

Version A1
For complete product history see page 6.

Catalogue numbers
- **D024-025R:** NXtec D024 KaryoProfiler, 25 reactions
- **D024-050R:** NXtec D024 KaryoProfiler, 50 reactions
- **D024-100R:** NXtec D024 KaryoProfiler, 100 reactions

NXtec D024-A1 KaryoProfiler (hereafter: D024 KaryoProfiler) 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))
   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 version Coffalyser digitalMLPA™ 2.5.0 or higher. (Cat No: n.a.)

Volumes and ingredients

Volumes			Ingredients
D024-025R	D024-050R	D024-100R	
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		

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 D024-A1 KaryoProfiler is a **research use only (RUO)** assay for the detection of gross copy number changes, encompassing all chromosomes, typically at a ~2-4 Mb resolution. Therefore, this probemix allows for fast and cost-effective genome-wide CN characterisation of DNA samples in a high-throughput fashion, allowing accurate detection of gross CN changes in various genetic backgrounds and contexts.

Possible applications may include the characterisation of DNA samples harbouring gross chromosomal abnormalities associated with genetic disorders. Such genetic aberrations may include gains or deletions of whole chromosomes, as well as gross copy number (CN) changes that are subchromosomal in nature (https://medlineplus.gov).

Gross CN determination is also pivotal in embryonic stem cell (ESC) and induced pluripotent stem cells (iPSC) characterisation, as these stem cells often acquire non-random genetic changes when cultured for a longer time. Alterations commonly include gains or losses of chromosomes or subchromosomal fragments. Frequently affected chromosomes include, but are not limited to, chromosomes 12, 17, 20 and X (The International Stem Cell Initiative, 2011; Assau et al, 2020).

Furthermore, additional applications of the NXtec D024-A1 KaryoProfiler may include:

- Follow-up analysis of abnormal findings from FISH, array CGH, or NGS.
- Complementation of karyotyping in cases with unclear or failed results.
- Monitoring genomic drift and instability in long-term or high-passage cultures.
- Screening and selection of clones prior to functional studies.
- Detection of low-level mosaicism or subclones when present in ≥30% of the DNA sample.
- Indication of cross-contamination, detecting contaminating cell fractions as low as 7.5%.

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 1587 probes are included in D024-A1 KaryoProfiler, consisting of:
- 1180 probes detecting copy number alterations in a genome-wide fashion, typically at a ~2-4 Mb resolution. See the Probe Information File (PIF) and Figure 1 for more details.
- More than 160 control probes and fragments: these include probes for sample identification, cross contamination detection, 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.

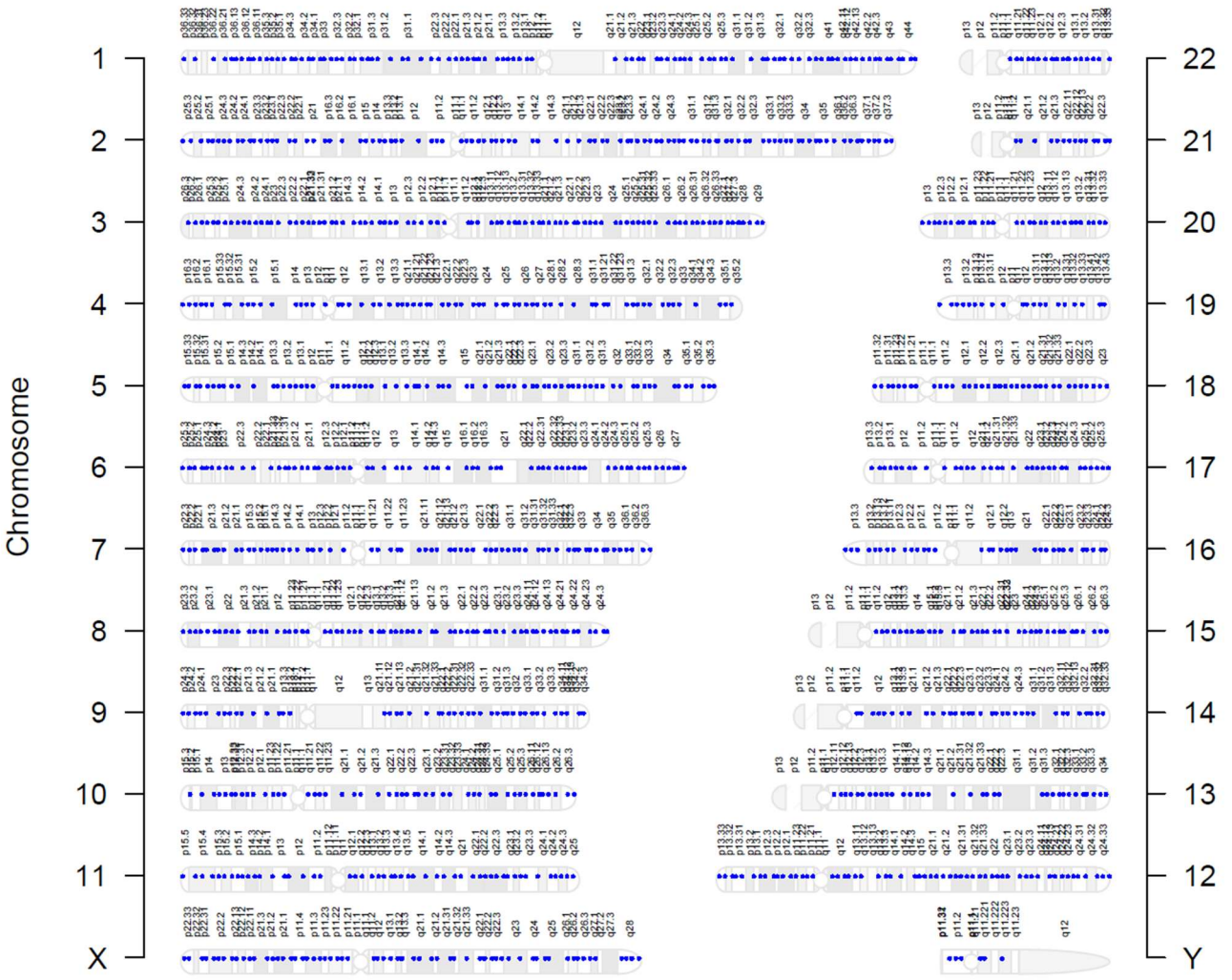

Figure 1: Chromosome plot showing the genomic loci that are covered by D024-A1 KaryoProfiler.
Each blue dot represents one target probe, with a total of 1180 target probes covering most chromosome arms, typically at a resolution of ~2-4 Mb. A complete overview of the probe locations can be found in the Probe Information File (PIF).

Reference probes
As the target probes are spread over a large number of different autosomal chromosomal regions, no separate reference probes have been included in D024 KaryoProfiler. Instead, a selection of 889 target probes is used as reference probe for data normalisation. The Probemix Information File (PIF) contains an overview of all the target probes that are present in D024-A1 KaryoProfiler, with reference probes indicated.

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 probes with the exception of SNP- and mutation-specific probes.

Required specimens
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)

For more information see the digitalMLPA NXtec Protocol, section DNA sample treatment.

Reference samples
A sufficient number (≥3) of different reference samples from unrelated individuals should be included in each digitalMLPA experiment for data normalisation. As X- and Y-chromosome specific probes are included in this probemix, at least three male reference samples need to be used per experiment. Please note that this applies **only** when Coffalyser digitalMLPA 2.5.0 or higher is used. In case an earlier software version is used, a different reference sample selection is needed, which includes at least three male AND three female reference samples. Pooled DNA from different sexes can never be used as reference samples for D024 KaryoProfiler 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.

When sufficient DNA samples from unrelated families are tested with D024-A1 KaryoProfiler, it is unlikely that the majority of the samples will have the same copy number change. In this case, using separate reference samples is not necessary and for data analysis using Coffalyser digitalMLPA the sample type should be set to "Test" (not "Reference") for all samples. The minimum number of required samples needs to be determined experimentally (read the background on our Support Portal).

However, when the testing sample set is small or includes many samples from the same family, or when tested samples are expected to have CNVs in the same genomic region(s), then inclusion of separate reference DNA samples in the experiment is required.

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. The Coriell Institute (https://catalog.coriell.org) has a diverse collection of biological resources which may be used as a positive control DNA sample in your digitalMLPA experiments. The quality of cell lines can change, therefore deviations to the indicated copy number variation (CNV) findings might occur. A list of positive control samples that have been tested with D024-A1 KaryoProfiler at MRC Holland can be found on the product page (https://www.mrcholland.com/product/D024).

Data analysis
Coffalyser digitalMLPA 2.5.0 or higher must be used for data analysis in combination with the latest version of the appropriate lot-specific product sheet. 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 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous duplication) or ≥4 (amplification). The same results can be expected for the X-chromosome-specific probes in female samples. For the X- and Y-chromosome-specific probes that are not in one of the pseudo-autosomal regions (PAR) in male samples, expected copy numbers are 1 (normal), 0 (deletion) or 2 (duplication).

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:

Copy number status		Inter ratio
(pseudo)autosomal sequences / X-chromosome sequences in female samples	**X- and Y-chromosome sequences in male samples**	
Normal	Normal	0.80 ≤ ratio ≤ 1.20
Homozygous deletion	Deletion	ratio = 0
Heterozygous deletion	-	0.40 < ratio < 0.65
Heterozygous duplication	-	1.30 < ratio < 1.65
Heterozygous triplication/ Homozygous duplication	Duplication	1.75 < ratio < 2.15
Ambiguous copy number	Ambiguous copy number	All other values*

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

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. single nucleotide variants (SNVs), point mutations) 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.

D024-A1 KaryoProfiler-specific notes

- This probemix is not suitable for genome-wide CN detection in DNA samples derived from tumour tissue, due to the genomic instability and more chaotic karyotypes often observed in tumours. High genomic instability and chaotic karyotypes may hamper data normalisation due to too many reference probes being subject to deviations, skewing the results by incorrect baseline determination.
- Detection of CNVs comprising one probe, or two adjacent probes, should always be confirmed by another method, as such deviations still have a considerable chance of being a false positive result due to genomic context (e.g. GC-rich region) in combination with suboptimal sample conditions (e.g. salt contamination) and sequence changes in the target sequence of the probe(s).
- The resolution of ~2-4 Mb is not completely maintained throughout the entire genome. For example, no probes are present in D024-A1 KaryoProfiler that target the p-arms of acrocentric chromosomes 13, 14, 15, 21 and 22. Pericentromeric regions of chromosomes 1, 9 and 16 are also not covered, as well as several other regions in the genome. Typically, exclusion of these regions is due to the absence of exonic sequences, or presence of complex or repetitive DNA sequences, known to disrupt digitalMLPA probe function.

Limitations of the procedure

- In certain cases, genetic defects may be caused by small (point) mutations, none of which will be detected by D024-A1 KaryoProfiler.

- 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.

Confirmation of results

Copy number changes of multiple consecutive probes detected with D024-A1 KaryoProfiler should be verified by another method when possible. MLPA probemixes are available for many chromosomal regions in D024-A1 KaryoProfiler. 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.

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

References

- Assou S et al. (2020). Recurrent Genetic Abnormalities in Human Pluripotent Stem Cells: Definition and Routine Detection in Culture Supernatant by Targeted Droplet Digital PCR. *Stem Cell Reports*. 14:1-8.
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- The International Stem Cell Initiative (2011). Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. *Nat Biotechnol*. 29:1132-44.
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D024 KaryoProfiler product history	
Version	*Modification*
A1	Not applicable.

Implemented changes in the product description
Version A1-02 – 10 November 2025 (05) - Modifications throughout the document to make this product description compatible with software version Coffalyser digitalMLPA 2.5.0 or higher, especially with regards to reference sample selection. - Positive control DNA sample section was updated to include a reference to the product page. Version A1-01 – 11 November 2025 (05) - Not applicable, new document.

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