

MiSeq™ Dx Reagent Kit v3

FOR IN VITRO DIAGNOSTIC USE

Intended Use

The Illumina MiSeqDx Reagent Kit v3 is a set of reagents and consumables intended for sequencing of sample libraries when used with validated assays. The MiSeqDx Reagent Kit v3 is intended for use with the MiSeqDx instrument and analytical software.

Principles of Procedure

For its input, the MiSeqDx Reagent Kit v3 uses libraries prepared from DNA where sample indexes and capture sequences are added to targets. Sample libraries are captured on a flow cell and sequenced on the instrument using sequencing by synthesis (SBS) chemistry. SBS chemistry uses a reversible-terminator method to detect fluorescently-labeled single nucleotide bases as they are incorporated into growing DNA strands.

For instructions for performing sequencing on the MiSeqDx instrument, see the MiSeqDx Instrument Package Insert for your region and version of the instrument software.

Limitations of the Procedure

- For *in vitro* diagnostic use.
- Limitations described in this package insert are based on representative assays and software modules, including the Local Run Manager Germline Variant and Somatic Variant Modules, which were developed for the purpose of evaluating performance with representative assays.
- Reads containing insertions, deletions, or combinations thereof (indels).
Content of length > 25 bp is not aligned by the assay software. Consequently, indels of length > 25 base pairs (bp) are not detectable by the assay software.
- The system has been validated for the detection of single nucleotide variants (SNVs) and up to 25 bp deletions and 24 bp insertions when used with the Germline and Somatic Variant module software. For somatic calling, at a variant frequency of 0.05, 25 bp deletions and 18 bp insertions were detected.
- The assay software might not align amplicon reads with extreme variant content, resulting in the region being reported as wild-type. Such extreme content includes:

- Reads containing more than three indels
- Reads of length at least 30 bp with SNV content > 4% of the total amplicon target length (excluding probe regions)
- Reads of length < 30 bp with SNV content > 10% of the total amplicon length (including probe regions)
- Large variants, including multi-nucleotide variants (MNVs) and large indels, might be reported as separate smaller variants in the output VCF file.
- Deletion variants can be filtered or missed when spanning two tiled amplicons if the deletion length is greater than or equal to the overlap between the tiled amplicons.
- The system cannot detect indels if they occur directly adjacent to a primer and there is no overlapping amplicon. For regions with overlapping amplicons, the assay cannot detect deletions when the region of overlap is smaller than the size of deletion to be detected. For example, if the region of overlap between two adjacent amplicons is two bases, the assay cannot detect any deletions including both of those bases. A single base deletion at either of those bases can be detected.
- As with any hybridization-based library preparation workflow, underlying polymorphisms, mutations, insertions, or deletions in oligonucleotide-binding regions can affect the alleles being probed and, consequently, the calls made during sequencing. For example:
 - A variant in phase with a variant in the primer region might not be amplified resulting in a false negative.
 - Variants in the primer region could prevent the amplification of the reference allele, resulting in an incorrect homozygous variant call.
 - Indel variants in the primer region can cause a false positive call at the end of the read adjacent to the primer.
- Indels can be filtered due to strand bias if they occur near the end of one read and are soft-clipped during alignment.
- Small MNVs have not been validated and are only reported in the Somatic Variant Module.
- Deletions are reported in the VCF at the coordinate of the preceding base per VCF format. Therefore, consider adjacent variants before reporting that an individual base call is homozygous reference.
- Germline-specific limitations:
 - The MiSeqDx instrument, using the Local Run Manager Germline Variant Module, is designed to deliver qualitative results for germline variant calling (eg, homozygous, heterozygous, wild-type).
 - When used with the Germline Variant Module, the minimum coverage per amplicon needed for accurate variant calling is 150x. As a result, 150 supporting DNA fragments are required, which

is equivalent to 300 overlapping paired-end reads. The number of samples and the total number of bases targeted affect coverage. GC-content and other genomic content can affect coverage.

- Copy number variation can affect whether a variant is identified as homozygous or heterozygous.
- Variants in certain repetitive context are filtered out in the VCF files. The RMxN repeat filter is used to filter variants if all or part of the variant sequence is present repeatedly in the reference genome adjacent to the variant position. For germline variant calling, at least nine repeats in the reference are required for a variant to be filtered, and only repeats with length up to 5 bp are considered (R5x9).
- Somatic-specific limitations:
 - The MiSeqDx instrument, using the Local Run Manager Somatic Variant Module, is designed to deliver qualitative results for somatic variant calling (eg, presence of a somatic variant with a variant frequency ≥ 0.026 with a limit of detection of 0.05).
 - When used with the Somatic Variant Module, the minimum coverage per amplicon needed for accurate variant calling is 450x per oligonucleotide pool. As a result, 450 supporting DNA fragments are required per oligonucleotide pool, which is equivalent to 900 overlapping paired-end reads. The number of samples and the total number of bases targeted affect coverage. GC-content and other genomic content can affect coverage.
 - For somatic variant calling, at least six repeats in the reference are required for the variant to be filtered, and only repeats with length up to 3 bp are considered (R3x6).
 - The Somatic Variant Module cannot differentiate between germline and somatic variants. The module is designed to detect variants across a range of variant frequencies, but variant frequency cannot be used to differentiate somatic variants from germline variants.
 - Normal tissue in the specimen impacts the detection of variants. The reported limit of detection is based on a variant frequency relative to the total DNA extracted from both tumor and normal tissue.

Product Components

Illumina MiSeqDx Reagent Kit v3, Catalog # 20037124 consists of the following:

- Library dilution buffer, flow cell, and post-amp reagents

Reagents Provided

The Illumina MiSeqDx Reagent Kit v3 is a single-use set of reagents and consumables for sequencing one run of one or more sample libraries on the MiSeqDx instrument. The number of sample libraries depends on the multiplexing supported by the upstream library preparation method.

See the following tables for a complete list of reagents provided in this kit.

MiSeqDx Reagent Kit v3, Box 1

Table 1 Box 1 Post-Amp Reagents

Component	Quantity	Fill Volume	Active Ingredients	Storage
Library Dilution Buffer	1 tube	4.5 ml	Buffered aqueous solution	-25°C to -15°C
MiSeqDx Reagent v3 Cartridge (RFID-labeled)	1 each	Various	Single-use prefilled cartridge	-25°C to -15°C

MiSeqDx Reagent Kit v3, Box 2

Table 2 Box 2 Post-Amp Reagents

Component	Quantity	Fill Volume	Active Ingredients	Storage
MiSeqDx SBS Solution (PR2) (RFID-labeled)	1 bottle	500 ml	Buffered aqueous solution	2°C to 8°C
MiSeqDx Flow Cell (RFID-labeled)	1 each	N/A	Single-use, paired-end, glass flow cell in buffered aqueous solution	2°C to 8°C

Storage and Handling

- Room temperature is defined as 15°C to 30°C.

- The following reagents are shipped frozen and are stable when stored at -25°C to -15°C until the specified expiration date.
 - Library Dilution Buffer
 - MiSeqDx Reagent v3 Cartridge

NOTE: The Library Dilution Buffer and MiSeqDx Reagent v3 Cartridge are single-use only and are stable for a maximum of one thaw to room temperature before the specified expiration date. Once thawed, the reagent cartridge must be loaded with sample library and immediately run on the MiSeqDx instrument. Alternatively, the thawed reagent cartridge can be kept at 2°C to 8°C for up to 6 hours, then loaded with sample library and immediately run on the MiSeqDx instrument.

- The following reagents are shipped refrigerated and are stable when stored at 2°C to 8°C until the specified expiration date.
 - MiSeqDx SBS Solution (PR2)
 - MiSeqDx Flow CellMiSeqDx SBS Solution (PR2) and MiSeqDx Flow Cell are single-use only.
- Changes in the physical appearance of the reagents can indicate deterioration of the materials. If changes in the physical appearance occur (eg, obvious changes in reagent color or cloudiness apparent with microbial contamination), do not use the reagents.

Equipment and Materials Required, Sold Separately

- **MiSeqDx Instrument**, Catalog # DX-410-1001

Warnings and Precautions



CAUTION

Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he/she practices, to use or order the use of the device.



WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the safety data sheet (SDS) at support.illumina.com/sds.html.

(See Reagents on page 1 for more information.)

- Failure to follow the procedures as outlined may result in erroneous results or significant reduction in sample quality.
- Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and assay reagents. Wash hands thoroughly after handling specimens and assay reagents.
- Proper laboratory practices and good laboratory hygiene are required to prevent PCR products from contaminating reagents, instrumentation, and genomic DNA samples. PCR contamination may cause inaccurate and unreliable results.
- To prevent contamination, ensure that pre-amplification and post-amplification areas have dedicated equipment (eg, pipettes, pipette tips, vortexer, and centrifuge).
- Index-sample pairing must match the printed plate layout exactly. Local Run Manager automatically populates the index primers associated with the sample names, when entered in the module. Verify the index primers associate with samples before starting the sequencing run. Mismatches between the printed plate layout and the sample will result in loss of positive sample identification and incorrect result reporting.
- Immediately report any serious incidents related to this product to Illumina and the Competent Authority of the Member State in which the user and/or patient is established.

Instructions for Use

See the MiSeqDx Instrument Package Insert for your region and version of the instrument software.

Performance Characteristics

See the MiSeqDx Instrument Package Insert for your region and version of the instrument software.

Revision History

Document #	Date	Description of Change
Document # 1000000030849 v04	November 2021	Updated MiSeqDx Instrument Package Insert references. Updated Limitations of the Procedure and Warnings and Precautions with additional clarifications. Minor updates to align with Illumina documentation style and standards.
Document # 1000000030849 v03	August 2021	Updated EU Authorized Representative address. Added Revision History table.

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Product Labeling

For a complete reference to symbols that may appear on product packaging and labeling, refer to the symbol key for your kit at support.illumina.com.