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# XCeloSeq® Breast Cancer cfDNA Kit V2

**SEQ033**

## Product Description

This kit contains reagents for the capture and subsequent independent targeted enrichment of both the sense and anti-sense DNA strands of mutation hotspots from 12 genes frequently mutated in breast cancers. The workflow uses cell-free DNA as starting material and, in combination with the second generation of the ATOM-Seq chemistry, allows for the generation of high quality, high-complexity next-generation sequencing libraries that are suitable for use with Illumina® next-generation sequencing instruments.

Please refer to **XCeloSeq Targeted cfDNA Enrichment V2 – Protocol** (IFU2115) for detailed instructions for use. A Laboratory Protocol is available for use in the laboratory to track and record completion of the protocol, **XCeloSeq Targeted cfDNA Enrichment V2 - Laboratory Protocol** (IFU2244).

## Assay Targets

Selected hotspots and target regions are enriched from within the following genes. Target region bed files are available upon request.

| Gene | Accession(s) | Exon(s) | Targets |
|---|---|---|---|
| AKT1 | NM_005163.2 | 3, 6 | Hotspots |
| CCND1 | NM_053056.3 | 4, 5 | Hotspots |
| EGFR | NM_005228.5 | 2, 3, 6, 7, 8, 10, 11, 12, 15, 18, 19, 20, 21, 22, 24, 26 | Hotspots |
| ERBB2 | NM_004448.4 | 2, 3, 4, 5, 7, 8, 17, 18, 19, 20, 21, 22, 24 | Hotspots |
| ERBB3 | NM_001982.4 | 3, 7, 8, 9, 23 | Hotspots |
| ESR1 | NM_000125.4 | 2, 5, 7, 8 | Hotspots |
| FBXW7 | NM_018315.5 | 4, 6, 7, 8, 9, 10, 11 | Hotspots |
| FGFR1 | NM_015850.4 | 4, 5, 7, 9, 10, 11, 13, 14, 15, 16, 18 | Hotspots |
| KRAS | NM_004985.5 | 2, 3, 4 | Hotspots |
| PIK3CA | NM_006218.4 | 2, 3, 5, 6, 7, 8, 10, 12, 14, 19, 21 | Hotspots |
| SF3B1 | NM_012433.4 | 14, 15 | Hotspots |
| TP53 | NM_000546.6<br>NM_001126113.3<br>NM_001126114.3 | 2, 3, 4, 5, 6, 7, 8, 9, 10<br>2, 3, 4, 5, 6, 7, 8, 9, 10<br>2, 3, 4, 5, 6, 7, 8, 9, 10 | Whole coding region +/-2 2bp |

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## Kit Contents

Upon receipt the kit will consist of three boxes.

| Box | Box name | REF ID | Storage (°C) |
|---|---|---|---|
| A | XCeloSeq Breast Cancer cfDNA Kit V2 | SEQ033 | -20 |
| B | XCeloSeq Targeted cfDNA Core Reagents V2 (Box 1 of 2) | GF020-V2 | -20 |
| C | XCeloSeq Targeted cfDNA Core Reagents V2 (Box 2 of 2) | GF020-BDX | 2-10 |

Box 1 contains target enrichment primers specific to the Breast Cancer cfDNA Kit V2-01 (see table below).

| Component name | Cap colour | Storage | Component REF |
|---|---|---|---|
| Pool 1 – Outer | Orange | -20°C | PC0704 |
| Pool 1 – Inner | Black | -20°C | PC0705 |
| Pool 2 – Outer | White | -20°C | PC0706 |
| Pool 2 – Inner | Yellow | -20°C | PC0707 |

Boxes 2 and 3 contain the core reagents which are universal reagents used across the whole range of XCeloSeq cfDNA enrichment kits. Please see the XCeloSeq Targeted cfDNA Enrichment V2 – Protocol for detailed contents.

## Kit Specifications and Recommendations

| | | |
|---|---|---|
| **Gene targets** | 12 | |
| **Targeting primers[%]** | Pool 1: 122<br>Pool 2: 119 | |
| **Supported input material** | Cell-free DNA | |
| **Input quantity** | Recommended: 30 - 50 ng<br>Minimum: 1 ng<br><br>Larger quantities will improve maximum sensitivity | |
| **Protocol duration** | Hands-on Time | 1.5 hours |
| | Total Protocol Time | 6 hours |

% Targeting primers are split between pool 1 which enriches sense DNA and pool 2 which enriches antisense DNA.

*Higher quantities will improve maximum sensitivity.

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## Sequencing Requirements

Libraries are natively compatible with Illumina sequencers, below are specifications for the index length and the recommended read length.

| | | |
|---|---|---|
| **Technical sequencing requirements** | Indexes | Dual 8 bp index |
| | Read length | 150 bp paired-end |

The number of captured DNA molecules from the original starting sample is proportional to both i) mass of input cfDNA and ii) the total depth of sequencing. Therefore, relatively deep sequencing is necessary to provide sufficient sequencing to allow all of the UMIs and all of the captured DNA molecules to be represented in the sequencing data.

The below table provides guidance on recommended sequencing depths for a range of starting cfDNA input masses. Sequencing depths can be adjusted based on user requirements and optimisations.

Sequencing must be equally divided between the Pool 1 and Pool 2 libraries generated by the workflow to achieve the maximum sensitivity for the protocol. A single "Paired Read" consists of a pair between a Read 1 and Read 2 generated during paired-end sequencing.

| **Sequencing requirements** | **Cell-free DNA input mass** | | | | | |
|---|---|---|---|---|---|---|
| | **1-10 ng** | | **10-30 ng** | | **30< ng** | |
| Recommended read pairs per primer | 7,500x | | 15,000x | | 30,000x | |
| Recommended read pairs per sample | Total: 1.9 M | | Total: 3.8 M | | Total: 7.6 M | |
| | Pool 1: 0.95 M | Pool 2: 0.95 M | Pool 1: 1.9 M | Pool 2: 1.9 M | Pool 1: 3.8 M | Pool 1: 3.8 M |

If one or both pools receives too few sequencing reads, the maximum sensitivity of the final data analysis will be reduced.

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The number of samples which can be multiplexed on a single sequencing run is dependent upon the size of the panel being used, the necessary depth per sample, and the capacity of the sequencing platform being used.

Below are guidelines for the number of samples processed using the Breast Cancer cfDNA Kit V2-01 which can be multiplexed on different sequencing platforms.

| | | Samples per sequencing run, for various cell-free DNA input masses | | |
|---|---|---|---|---|
| **Illumina instrument*** | **Version** | **1-10 ng** | **10-30 ng** | **>30 ng** |
| MiSeq | v2 Reagents | 8 | 4 | 2 |
| | v3 Reagents | 13 | 6 | 3 |
| MiSeq i100 | 5M | 2 | 1 | - |
| | 25M | 13 | 6 | 3 |
| | 50M | 27 | 13 | 6 |
| | 100M | 55 | 27 | 13 |
| NextSeq 550 | Mid output | 71 | 35 | 17 |
| | High output | 221 | 110 | 55 |
| NextSeq 1000/2000 | P1 | 55 | 27 | 13 |
| | P2 | 221 | 110 | 55 |
| NextSeq 2000 | P3 | 663 | 331 | 165 |
| NovaSeq 6000 | SP (2 lanes per flow cell) | 442 | 221 | 110 |
| | S1 (2 lanes per flow cell) | 885 | 442 | 221 |
| | S2 (2 lanes per flow cell) | 2268 | 1134 | 567 |
| | S3 (4 lanes per flow cell) | 5532 | 2766 | 1383 |

*Please see Illumina's website for detailed instrument specifications

## Additional Information

If you have any questions regarding this kit or the suitability of your samples, please contact customer support at sales@genefirst.com

## Limitations of Use

**For Research Use Only (RUO).**

This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals. SDS sheets relevant to this product are available upon request.



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