

XCeloSeq[®] Colon Cancer cfDNA Kit V2

SEQ031

Product Description

This kit contains reagents for the capture and subsequent independent targeted enrichment of both the sense and anti-sense DNA strands of 23 genes frequently mutated in Colon Cancer. 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
AMER1	NM_152424.4	2	Hotspots
APC	NM_000038.6	16	Hotspots
ARAF	NM_001654.5	7, 10, 11, 14	Hotspots
BRAF	NM_004333.6	8, 11, 14, 15, 16, 36, 37	Hotspots
CTNNB1	NM_001904.4	3, 7, 8, 11	Hotspots
DMD	NM_000109.4	3, 6, 10, 19, 26, 37, 52, 53, 54, 56, 57, 59,61,64, 65, 66, 68	Hotspots
DPYD	NM_000110.4	rs67376798, DPYD*A2 (rs3918290), rs55886062, HapB3 (rs75017182, rs56038477)	Genomic SNPs
EP300	NM_001429.4	18, 225, 26, 27, 28, 30	Hotspots
ERBB3	NM_001982.4	3, 7, 8, 9, 23	Hotspots
FBXW7	NM_018315.5	4, 6, 7, 8, 9, 10, 11	Hotspots
FGFR4	NM_002011.5	12, 13	Hotspots
GNAS	NM_000516.7	8, 9	Hotspots
HRAS	NM_005343.4	2, 3, 4	Hotspots
KRAS	NM_004985.5	2, 3, 4	Hotspots

Gene	Accession(s)	Exon(s)	Targets
MAP2K1	NM_002755.4	2, 3, 6	Hotspots
MAP2K2	NM_030662.4	2, 6	Hotspots
NRAS	NM_002524.5	2, 3, 4	Hotspots
PDGFRA	NM_006206.6	7, 10, 11, 12, 14, 15, 16, 18	Hotspots
PIK3CA	NM_006218.4	2, 3, 5, 6, 7, 8, 10, 12, 14, 19, 21	Hotspots
ROS1	NM_002944.3	36, 37, 38, 39, 42	Hotspots
SMAD4	NM_005359.6	2, 3, 4, 5, 6, 8, 9, 10, 11, 12	Hotspots
TCF7L2	NM_030756.5	10	Hotspots
TP53	NM_000546.6	2, 3, 4, 5, 6, 7, 8, 9, 10	Whole coding region +/-2 2bp
	NM_001126113.3	2, 3, 4, 5, 6, 7, 8, 9, 10	
	NM_001126114.3	2, 3, 4, 5, 6, 7, 8, 9, 10	

Kit Contents

Upon receipt the kit will consist of three boxes.

Box	Box name	REF ID	Storage (°C)
A	XCeloSeq Colon Cancer cfDNA Kit V2	SEQ031	-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 Colon Cancer cfDNA Kit V2 (see table below).

Component name	Cap colour	Storage (°C)	Component REF
Pool 1 – Outer	Orange	-20	PC0696
Pool 1 – Inner	Black	-20	PC0697
Pool 2 – Outer	White	-20	PC0698
Pool 2 – Inner	Yellow	-20	PC0699

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 and Protocol Specifications

Gene targets	23	
Targeting primers%	Pool 1: 155 Pool 2: 156	
Supported input material	Cell-free DNA	
Input quantity	Recommended: 30 - 50 ng Minimum: 1 ng 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.

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 per sample					
	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: 2.4 M		Total: 4.8 M		Total: 9.4 M	
	Pool 1: 1.2 M	Pool 2: 1.2 M	Pool 1: 2.4 M	Pool 2: 2.4 M	Pool 1: 4.7 M	Pool 1: 4.7 M

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

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 Colon Cancer cfDNA Kit V2 which can be multiplexed on different sequencing platforms.

Illumina instrument*	Version	Samples per sequencing run, for various cell-free DNA input masses		
		1-10 ng	10-30 ng	>30 ng
MiSeq	v2 Reagents	6	3	1
	v3 Reagents	10	5	2
MiSeq i100	5M	2	1	-
	25M	10	5	2
	50M	21	10	5
	100M	42	21	10
NextSeq 550	Mid output	54	27	13
	High output	168	84	42
NextSeq 1000/2000	P1	42	21	10
	P2	168	84	42
NextSeq 2000	P3	504	252	126
NovaSeq 6000	SP (2 lanes per flow cell)	336	168	84
	S1 (2 lanes per flow cell)	672	336	168
	S2 (2 lanes per flow cell)	1724	862	431
	S3 (4 lanes per flow cell)	4206	2103	1051

*Please see Illumina's website for detailed instrument specifications and availability



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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