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Product Insert

XCeloSeq® Pan Cancer cfDNA Kit V2

SEQ030

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 100 genes frequently mutated across 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 target hotspots are enriched from within the following genes. Target region bed files are available upon request.

Gene	Accession(s)	Exon(s)	Targets
ABL1	NM_005157.6	4, 5, 6, 7, 8	Hotspots
AKT1	NM_005163.2	3, 6	Hotspots
ALK	NM_004304.5	21, 22, 23, 24, 25	Hotspots
AMER1	NM_152424.4	2	Hotspots
APC	NM_000038.6	16	Hotspots
AR	NM_000044.6	8	Hotspots
ARAF	NM_001654.5	7, 10, 11, 14	Hotspots
ARID1A	NM_006015.6	14, 20	Hotspots
ATM	NM_000051.4	2, 7, 8, 9, 12, 17, 26, 34, 35, 36, 39, 45, 50, 54, 55, 56, 58, 59, 61, 62, 63	Hotspots
BRAF	NM_004333.6	8, 11, 14, 15, 16	Hotspots
BRCA1	NM_007294.4	10, 21, 23	Hotspots
BRCA2	NM_000059.4	7, 11, 13, 15, 16, 17, 18, 19, 20, 21, 24, 25	Hotspots
CASP8	NM_001228.5	3, 8, 9	Hotspots
CCND1	NM_053056.3	4, 5	Hotspots
CCND2	NM_001759.4	2, 3, 4, 5	Hotspots
CCND3	NM_001760.5	2, 3, 4, 5	Hotspots
CDH1	NM_004360.5	3, 6, 8, 9, 14	Hotspots
CDK4	NM_000075.4	2, 3, 4, 5, 6, 8	Hotspots

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CDK6	NM_001259.8	3, 4, 5, 7	Hotspots
CDKN2A	NM_000077.5	1, 2	Hotspots
CHEK2	NM_007194.4	11	Hotspots
CSF1R	NM_005211.4	7, 22	Hotspots
CTNNB1	NM_001904.4	3, 7, 8, 11	Hotspots
DDR2	NM_006182.4	8, 15, 17	Hotspots
DMD	NM_000109.4	3, 6, 10, 19, 26, 37, 52, 53, 54, 56, 57, 59, 61, 64, 65, 66, 68	Hotspots
EGFR	NM_000110.4	2, 3, 6, 7, 8, 10, 11, 12, 15, 18, 19, 20, 21, 22, 24, 26	Hotspots
EP300	NM_005228.5	18, 25, 26, 27, 28, 30	Hotspots
ERBB2	NM_001429.4	2, 3, 4, 5, 7, 8, 17, 18, 19, 20, 21, 22, 24	Hotspots
ERBB3	NM_004448.4	3, 7, 8, 9, 23	Hotspots
ERBB4	NM_001982.4	4, 6, 7, 9, 15, 18, 23	Hotspots
ESR1	NM_005235.3	2, 5, 7, 8	Hotspots
EZH2	NM_000125.4	5, 7, 16	Hotspots
FBXW7	NM_004456.5	4, 6, 7, 8, 9, 10, 11	Hotspots
FGFR1	NM_018315.5	4, 5, 7, 9, 10, 11, 13, 14, 15, 16, 18	Hotspots
FGFR2	NM_015850.4	2, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16	Hotspots
FGFR3	NM_000141.5	4, 7, 8, 9, 13, 14, 16	Hotspots
FGFR4	NM_000142.5	13	Hotspots
FLT3	NM_002011.5	11, 13, 14, 15, 16, 19, 20, 21, 23	Hotspots
GATA3	NM_004119.3	3, 4, 5, 6	Hotspots
GNA11	NM_002051.3	4, 5	Hotspots
GNAQ	NM_002067.5	4, 5	Hotspots
GNAS	NM_002072.5	8, 9	Hotspots
HNF1A	NM_000516.7	3, 4, 6	Hotspots
HRAS	NM_000545.8	2, 3, 4	Hotspots
IDH1	NM_005343.4	4	Hotspots
IDH2	NM_005896.4	4	Hotspots
JAK2	NM_002168.4	14, 16	Hotspots
JAK3	NM_004972.4	4, 12, 13, 14, 15, 16, 17, 20, 21, 24	Hotspots
KDM6A	NM_000215.4	14, 17, 21, 22, 23, 25, 26	Hotspots
KDR	NM_021140.4	6, 7, 11, 19, 21, 26, 27, 30	Hotspots
KEAP1	NM_002253.4	2, 3, 4	Hotspots
KIT	NM_012289.4	2, 8, 9, 10, 11, 13, 14, 15, 17, 18, 21	Hotspots
KLF5	NM_000222.3	4	Hotspots
KRAS	NM_001730.5	2, 3, 4	Hotspots
MAP2K1	NM_004985.5	2, 3, 6	Hotspots
MAP2K2	NM_002755.4	2, 6	Hotspots

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Gene	Accession(s)	Exon(s)	Targets
MET	NM_030662.4	2, 3, 4, 11, 14, 16, 17, 18, 19, 20	Hotspots
MGA	NM_000245.4	19	Hotspots
MLH1	NM_001080541.3	2, 8, 12	Hotspots
MPL	NM_000249.4	10	Hotspots
MSH2	NM_005373.3	3, 4, 6, 7, 12, 13, 14	Hotspots
MSH6	NM_000251.3	4, 5, 6, 9	Hotspots
MTOR	NM_000179.3	30, 39, 40, 43, 44, 45, 47, 48, 50, 53, 56	Hotspots
MYC	NM_004958.4	1, 2, 3	Hotspots
NF1	NM_002467.6	21, 28, 37, 49	Hotspots
NFE2L2	NM_000267.3	2	Hotspots
NOTCH1	NM_006164.5	8, 26, 27, 34	Hotspots
NPM1	NM_017617.5	3, 11	Hotspots
NRAS	NM_002520.7	2, 3, 4	Hotspots
NTRK1	NM_002524.5	14, 15	Hotspots
NTRK3	NM_002529.4	16	Hotspots
PDGFRA	NM_002530.4	7, 10, 11, 12, 14, 15, 16, 18	Hotspots
PIK3CA	NM_006206.6	2, 3, 5, 6, 7, 8, 10, 12, 14, 19, 21	Hotspots
PTCH1	NM_006218.4	5, 7, 8, 11, 12, 13, 14, 16, 17, 18, 21	Hotspots
PTEN	NM_000264.5	1, 2, 3, 4, 5, 6, 7, 8, 9	Hotspots
PTPN11	NM_000314.8	3, 13	Hotspots
RAF1	NM_002834.5	7, 10, 17	Hotspots
RB1	NM_002880.4	2, 3, 4, 6, 10, 11, 13, 14, 17, 18, 20, 21, 22, 23, 25	Hotspots
RBM10	NM_000321.3	18, 20, 23	Hotspots
RET	NM_005676.5	10, 11, 13, 14, 15, 16	Hotspots
RHOA	NM_020630.7	2, 3, 5	Hotspots
RIT1	NM_001664.4	5	Hotspots
RNF43	NM_006912.6	8	Hotspots
ROS1	NM_017763.6	36, 37, 38, 39, 42	Hotspots
SETD2	NM_002944.3	7	Hotspots
SF3B1	NM_014159.7	15	Hotspots
SMAD2	NM_012433.4	4, 8	Hotspots
SMAD4	NM_005901.6	2, 3, 4, 5, 6, 8, 9, 10, 11, 12	Hotspots
SMARCA4	NM_005359.6	16, 17, 19, 20, 25, 26	Hotspots
SMARCB1	NM_003072.5	2, 4, 5, 9	Hotspots
SMO	NM_003073.5	3, 5, 6, 8, 9, 11	Hotspots
SRC	NM_005631.5	14	Hotspots
STK11	NM_005417.5	1, 4, 5, 6, 7, 8, 9	Hotspots
TCF7L2	NM_000455.5	10	Hotspots
TP53	NM_030756.5	2, 3, 4, 5, 6, 7, 8, 9, 10, 11	Whole coding region +/-2 2bp

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Gene	Accession(s)	Exon(s)	Targets
TSC1	NM_000368.5	4, 6, 7, 8, 9, 15, 16, 17	Hotspots
TSC2	NM_000548.5	6, 12, 15, 23, 30, 36, 40	Hotspots
U2AF1	NM_006758.3	2, 6, 7, 8	Hotspots
VHL	NM_000551.4	1, 2, 3	Hotspots
ZFP36L2	NM_006887.5	2	Hotspots

Kit Contents

Upon receipt the kit will consist of three boxes:

Box	Box name	Box REF	Storage (°C)
A	XCeloSeq Pan Cancer cfDNA Kit V2	SEQ030	-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 A contains target enrichment primers specific to the Pan Cancer cfDNA Kit V2:

Component name	Cap colour	Storage (°C)	Component REF
Pool 1 – Outer	Orange	-20	PC0692
Pool 1 – Inner	Black	-20	PC0693
Pool 2 – Outer	White	-20	PC0694
Pool 2 – Inner	Yellow	-20	PC0695

Boxes B and C 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.

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Kit and Protocol Specifications

Gene targets	100	
Targeting primers[%]	Pool 1: 577 Pool 2: 570	
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.

*Higher quantities will improve maximum sensitivity.

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) the 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 “Read Pair” consists of a pair between a Read 1 and Read 2 generated from a single cluster during paired-end sequencing.

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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: 8.8 M		Total: 17.6 M		Total: 35.2 M	
	Pool 1: 4.4 M	Pool 2: 4.4 M	Pool 1: 8.8 M	Pool 2: 8.8 M	Pool 1: 17.6 M	Pool 1: 17.6 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 Pan 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	1	-	-
	v3 Reagents	2	1	-
MiSeq i100	5M	-	-	-
	25M	2	1	-
	50M	5	2	1
	100M	11	5	2
NextSeq 550	Mid output	15	7	3
	High output	46	23	11
NextSeq 1000/2000	P1	11	5	2
	P2	46	23	11
NextSeq 2000	P3	139	69	34
NovaSeq 6000	SP (2 lanes per flow cell)	92	46	23
	S1 (2 lanes per flow cell)	185	92	46
	S2 (2 lanes per flow cell)	476	238	119
	S3 (4 lanes per flow cell)	1162	581	290

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

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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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