Product Insert

XCeloSeq® Lung Cancer cfDNA Kit V2

SEQ032

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 17 genes frequently mutated in Lung 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 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
ALK	NM_004304.5	21, 22, 23, 24, 25	Hotspots
BRAF	NM_004333.6	8, 11, 14, 15, 16, 36, 37	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
ESR1	NM_000125.4	2, 5, 7, 8	Hotspots
HRAS	NM_005343.4	2, 3, 4	Hotspots
KEAP1	NM_012289.4	2, 3, 4	Hotspots
KRAS	NM_004985.5	2, 3, 4	Hotspots
MAP2K1	NM_002755.4	2, 3, 6	Hotspots
MET	NM_000245.4	2, 3, 4, 11, 14, 16, 17, 18, 19, 20	Hotspots
NRAS	NM_002524.5	2, 3, 4	Hotspots
PIK3CA	NM_006218.4	2, 3, 5, 6, 7, 8, 10, 12, 14, 19, 21	Hotspots
RET	NM_020630.7	10, 11, 13, 14, 15, 16	Hotspots
ROS1	NM_002944.3	36, 37, 38, 39, 42	Hotspots
STK11	NM_000455.5	1, 4, 5, 6, 7, 8, 9	Hotspots
TP53	NM_000546.6 NM_001126113.3 NM_001126114.3	2, 3, 4, 5, 6, 7, 8, 9, 10 2, 3, 4, 5, 6, 7, 8, 9, 10 2, 3, 4, 5, 6, 7, 8, 9, 10	Whole coding region +/-2 2bp

Kit Contents

Upon receipt the kit will consist of three boxes.

Box	Box name	REF ID	Storage (°C)
A	XCeloSeq Lung Cancer cfDNA Kit V2	SEQ032	-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 Lung Cancer cfDNA Kit V2-01 (see table below).

Component name	Cap colour	Storage (°C)	Component REF
Pool 1 – Outer	Orange	-20	PC0700
Pool 1 – Inner	Black	-20	PC0701
Pool 2 – Outer	White	-20	PC0702
Pool 2 – Inner	Yellow	-20	PC0703

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	17	
Targeting primers[%]	Pool 1: 150 Pool 2: 146	
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) 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.

	Cell-free DNA input mass					
Sequencing requirements	**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: 2.3 M		Total: 4.5 M		Total: 9.0 M	
	Pool 1: 1.15 M	Pool 2: 1.15 M	Pool 1: 2.25 M	Pool 2: 2.25 M	Pool 1: 4.5 M	Pool 1: 4.5 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 Lung Cancer cfDNA Kit V2-01 which can be multiplexed on different sequencing platforms.

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Product Insert

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	11	5	2
MiSeq i100	5M	2	1	-
	25M	11	5	2
	50M	22	11	5
	100M	45	22	11
NextSeq 550	Mid output	58	29	14
	High output	180	90	45
NextSeq 1000/2000	P1	45	22	11
	P2	180	90	45
NextSeq 2000	P3	540	270	135
NovaSeq 6000	SP (2 lanes per flow cell)	360	180	90
	S1 (2 lanes per flow cell)	720	360	180
	S2 (2 lanes per flow cell)	1846	923	461
	S3 (4 lanes per flow cell)	4504	2252	1126

*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.