Technical Specifications

FOUNDATIONONE®LIQUID CDx

Intended Use

FoundationOne Liquid CDx is a qualitative next generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology to detect and report substitutions,insertions and deletions (indels) in 311 genes, rearrangements in four (4) genes, and copy number alterations in three (3) genes. FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients collected in FoundationOne Liquid CDx cfDNA blood collection tubes included in the FoundationOne Liquid CDx Blood Sample Collection Kit. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1: Companion diagnostic indications

TUMOR TYPE	BIOMARKER(S) DETECTED	THERAPY
Non-small cell lung cancer (NSCLC)	*ALK* rearrangements	ALECENSA® (alectinib)
	EGFR Exon 19 deletions and *EGFR* Exon 21 L858R subsitution	IRESSA® (gefitinib) TAGRISSO® (osimertinib) TARCEVA® (erlotinib)
Prostate cancer	*BRCA1, BRCA2, ATM* alterations	LYNPARZA® (olaparib)
	BRCA1, BRCA2 alterations	RUBRACA® (rucaparib)
Ovarian cancer	*BRCA1, BRCA2* alterations	RUBRACA® (rucaparib)
Breast cancer	*PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y	PIQRAY® (alpelisib)

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Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms.

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients with the tumor types above who are negative for the mutations listed in Table 1 should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

FoundationOne Liquid CDx is a single-site assay performed at Foundation Medicine, Inc. in Cambridge, MA.

Summary of Analytical Sensitivity and Specificity for FDA-Approved Content

Results from our Limit of Detection (LoD) study are shown below, indicating the median variant allele frequency or tumor fraction at which the test has shown 95% probability of detection.[1] Results are also included from the Limit of Blank (LoB) study, which evaluated variant calling in healthy donors. Please refer to our product labeling for a list of the 75 genes baited for enhanced sensitivity are noted in bold in the gene list on the next page.

ALTERATION TYPE	BAIT SET REGION	MEDIAN LIMIT OF DETECTION (LOD)	LOD RANGE QUARTILE 1-3*	LIMIT OF BLANK (VARIANT DETECTION RATE IN HEALTHY DONORS)†
Short Variants	Enhanced Sensitivity	0.40% VAF	0.33% - 0.50% VAF	0.82%
	Standard Sensitivity	0.82% VAF	0.70% - 0.98% VAF	
Rearrangements	Enhanced Sensitivity	0.37% VAF	0.26% - 0.47% VAF	0%
	Standard Sensitivity	0.90% VAF	NA	
Copy Number Amplifications	NA	21.7% TF	19.8%-25.2% TF	0%

VAF = variant allele frequency; TF = tumor fraction

* The accuracy of %VAF / %TF have not been analytically validated

† Calculated as the number of unique variants detected at least once across all replicates divided by the total number of unique variants included in the analysis

FoundationOne Liquid CDx Gene List →

FoundationOne Liquid CDx FDA-Approved Gene List‡

As part of its FDA-approved intended use, FoundationOne Liquid CDx interrogates 311 genes, including 309 genes with complete exonic (coding) coverage and 2 genes with only select non-coding coverage (indicated with *). **Select genes and select exons (indicated in bold) are captured with increased sensitivity.**

ABL1 **Exons 4-9**	*ACVR1B*	***AKT1*** **Exon 3**	*AKT2*	*AKT3*	***ALK*** **Exons 20-29, Introns 18, 19**	*ALOX12B*
AMER1 (FAM123B)	***APC***	***AR***	***ARAF*** **Exons 4, 5, 7, 11, 13, 15, 16**	*ARFRP1*	*ARID1A*	*ASXL1*
ATM	***ATR***	*ATRX*	*AURKA*	*AURKB*	*AXIN1*	*AXL*
BAP1	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*
BCORL1	***BRAF*** **Exons 11-18**	***BRCA1*** Introns 2, 7, 8, 12, 16, 19, 20	***BRCA2*** Intron 2	*BRD4*	*BRIP1*	*BTG1*
BTG2	***BTK*** **Exons 2, 15**	*C11orf30 (EMSY)*	*C17orf39 (GID4)*	*CALR*	*CARD11*	*CASP8*
CBFB	*CBL*	***CCND1***	*CCND2*	*CCND3*	*CCNE1*	*CD22*
CD274 (PD-L1)	*CD70*	*CD79A*	*CD79B*	*CDC73*	***CDH1***	***CDK12***
CDK4	***CDK6***	*CDK8*	*CDKN1A*	*CDKN1B*	***CDKN2A***	*CDKN2B*
CDKN2C	*CEBPA*	*CHEK1*	***CHEK2***	*CIC*	*CREBBP*	***CRKL***
CSF1R	*CSF3R*	*CTCF*	*CTNNA1*	***CTNNB1*** **Exon 3**	*CUL3*	*CUL4A*
CXCR4	*CYP17A1*	*DAXX*	*DDR1*	***DDR2*** **Exons 5, 17, 18**	*DIS3*	*DNMT3A*
DOT1L	*EED*	***EGFR***	*EP300*	*EPHA3*	*EPHB1*	*EPHB4*
ERBB2	***ERBB3*** **Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25**	*ERBB4*	*ERCC4*	*ERG*	***ERRFI1***	***ESR1*** **Exons 4-8**
EZH2 **Exons 4, 16, 17, 18**	*FAM46C*	*FANCA*	*FANCC*	*FANCG*	*FANCL*	*FAS*
FBXW7	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*
FGF4	*FGF6*	***FGFR1***	***FGFR2***	***FGFR3*** **Exons 7, 9 (alternative designation exon 10), 14, 18**	*FGFR4*	*FH*
FLCN	*FLT1*	***FLT3*** **Exons 14, 15, 20**	***FOXL2***	*FUBP1*	*GABRA6*	*GATA3*
GATA4	*GATA6*	***GNA11*** **Exons 4, 5**	*GNA13*	***GNAQ*** **Exons 4, 5**	***GNAS*** **Exons 1, 8**	*GRM3*
GSK3B	*H3F3A*	*HDAC1*	*HGF*	*HNF1A*	***HRAS*** **Exons 2, 3**	*HSD3B1*
ID3	***IDH1*** **Exon 4**	***IDH2*** **Exon 4**	*IGF1R*	*IKBKE*	*IKZF1*	*INPP4B*
IRF2	*IRF4*	*IRS2*	*JAK1*	***JAK2*** **Exons 14**	***JAK3*** **Exons 5, 11, 12, 13, 15, 16**	*JUN*
KDM5A	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	***KIT*** **Exons 8, 9, 11, 12, 13, 17**
KLHL6	*KMT2A (MLL)*	*KMT2D (MLL2)*	***KRAS***	*LTK*	*LYN*	*MAF*
MAP2K1 (MEK1) **Exons 2, 3**	***MAP2K2 (MEK2)*** **Exons 2-4, 6, 7**	*MAP2K4*	*MAP3K1*	*MAP3K13*	*MAPK1*	*MCL1*
MDM2	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	***MET***
MITF	*MKNK1*	*MLH1*	***MPL*** **Exon 10**	*MRE11A*	*MSH2*	*MSH3*
MSH6	*MST1R*	*MTAP*	***MTOR*** **Exons 19, 30, 39, 40, 43-45, 47, 48, 53, 56**	*MUTYH*	***MYC***	*MYCL (MYCL1)*
MYCN	***MYD88*** **Exon 4**	*NBN*	***NF1***	*NF2*	*NFE2L2*	*NFKBIA*
NKX2-1	*NOTCH1*	*NOTCH2*	*NOTCH3*	***NPM1*** **Exons 4-6, 8, 10**	***NRAS*** **Exons 2, 3**	*NSD3 (WHSC1L1)*
NT5C2	***NTRK1*** **Exons 14, 15**	*NTRK2*	***NTRK3*** **Exons 16, 17**	*P2RY8*	***PALB2***	*PARK2*
PARP1	*PARP2*	*PARP3*	*PAX5*	*PBRM1*	*PDCD1 (PD-1)*	***PDCD1LG2 (PD-L2)***
PDGFRA **Exons 12, 18**	***PDGFRB*** **Exons 12-21, 23**	*PDK1*	*PIK3C2B*	*PIK3C2G*	***PIK3CA*** **Exons 2, 3, 5-8, 10, 14,1 9, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)**	*PIK3CB*
PIK3R1	*PIM1*	*PMS2*	*POLD1*	*POLE*	*PPARG*	*PPP2R1A*
PPP2R2A	*PRDM1*	*PRKAR1A*	*PRKCI*	*PTCH1*	***PTEN***	***PTPN11***
PTPRO	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*	*RAD51C*
RAD51D	*RAD52*	*RAD54L*	***RAF1*** **Exons 3, 4, 6, 7, 10, 14, 15, 17**	*RARA*	***RB1***	*RBM10*
REL	***RET*** **Exons 11, 13-16**	*RICTOR*	*RNF43*	***ROS1*** **Exons 31, 36-38, 40**	*RPTOR*	*SDHA*
SDHB	*SDHC*	*SDHD*	*SETD2*	*SF3B1*	*SGK1*	*SMAD2*
SMAD4	*SMARCA4*	*SMARCB1*	***SMO***	*SNCAIP*	*SOCS1*	*SOX2*
SOX9	*SPEN*	*SPOP*	*SRC*	*STAG2*	*STAT3*	***STK11***
SUFU	*SYK*	*TBX3*	*TEK*	*TERC** ncRNA	***TERT**** **Promoter**	*TET2*
TGFBR2	*TIPARP*	*TNFAIP3*	*TNFRSF14*	***TP53***	*TSC1*	*TSC2*
TYRO3	*U2AF1*	***VEGFA***	*VHL*	*WHSC1*	*WT1*	*XPO1*
XRCC2	*ZNF217*	*ZNF703*				

Summary of Analytical Sensitivity and Specificity for Professional Services Content

Results from our Limit of Detection (LoD) study are shown below, indicating the median variant allele frequency, tumor fraction or unstable loci at which the test has shown 95% probability of detection.[2] Please refer to our product labeling for a list of the 75 genes baited for enhanced sensitivity and complete product specifications.

ALTERATION TYPE	MEDIAN LIMIT OF DETECTION (LOD)
MSI	0.8% Unstable loci
bTMB (component indels)	1.00% VAF*
bTMB (component subs)	1.00% VAF*

VAF = variant allele frequency; TF = tumor fraction

* The accuracy of %VAF / %TF have not been analytically validated

In our Limit of Blank study, which evaluated variant calling in healthy donors, 1,735 unique variants were included in the analysis for a total of 137,065 data points. A total of 18 false positives were observed across 4 unique short variants. The LoB was determined to be the ideal value of zero for short variants, rearrangements and CNAs. The false positive rate was shown to be 0% for rearrangements and CNAs and 0.013% (~1 in 8,000) for short variants (substitutions and indels).[2]

Information Provided as a Professional Service

As a professional service, FoundationOne Liquid CDx interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an *); **75 genes (indicated in bold) are captured with increased sensitivity** and have complete exonic (coding) coverage unless otherwise noted. The test also detects tumor fraction and the genomic signatures blood mutational burden (bTMB) and microsatellite instability high (MSI-H) status.

ABL1 **Exons 4-9**	*ACVR1B*	***AKT1*** **Exon 3**	*AKT2*	*AKT3*	***ALK*** **Exons 20-29, Introns 18, 19**	*ALOX12B*
AMER1 (FAM123B)	***APC***	***AR***	***ARAF*** **Exons 4, 5, 7, 11, 13, 15, 16**	*ARFRP1*	*ARID1A*	*ASXL1*
ATM	***ATR***	*ATRX*	*AURKA*	*AURKB*	*AXIN1*	*AXL*
BAP1	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*
BCORL1	*BCR** Introns 8, 13, 14	***BRAF*** **Exons 11-18,** Introns 7-10	***BRCA1*** Introns 2, 7, 8, 12, 16, 19, 20	***BRCA2*** Intron 2	*BRD4*	*BRIP1*
BTG1	*BTG2*	***BTK*** **Exons 2, 15**	*C11orf30 (EMSY)*	*C17orf39 (GID4)*	*CALR*	*CARD11*
CASP8	*CBFB*	*CBL*	***CCND1***	*CCND2*	*CCND3*	*CCNE1*
CD22	*CD70*	*CD74** Introns 6-8	*CD79A*	*CD79B*	***CD274 (PD-L1)***	*CDC73*
CDH1	***CDK12***	***CDK4***	***CDK6***	*CDK8*	*CDKN1A*	*CDKN1B*
CDKN2A	*CDKN2B*	*CDKN2C*	*CEBPA*	*CHEK1*	***CHEK2***	*CIC*
CREBBP	***CRKL***	*CSF1R*	*CSF3R*	*CTCF*	*CTNNA1*	***CTNNB1*** **Exon 3**
CUL3	*CUL4A*	*CXCR*	*CYP17A1*	*DAXX*	*DDR1*	***DDR2*** **Exons 5, 17, 18**
DIS3	*DNMT3A*	*DOT1L*	*EED*	***EGFR*** **Introns 7,** 15**, 24-27**	*EP300*	*EPHA3*
EPHB1	*EPHB4*	***ERBB2***	***ERBB3*** **Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25**	*ERBB4*	*ERCC4*	*ERG*
ERRFI1	***ESR1*** **Exons 4-8**	*ETV4** Intron 8	*ETV5** Introns 6, 7	***ETV6**** **Introns 5, 6**	*EWSR1** Introns 7-13	***EZH2*** **Exons 4, 16, 17, 18**
*EZR** Introns 9-11	*FAM46C*	*FANCA*	*FANCC*	*FANCG*	*FANCL*	*FAS*
FBXW7	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*
FGF4	*FGF6*	***FGFR1*** Introns 1, 5, **Intron 17**	***FGFR2*** Intron 1, **Intron 17**	***FGFR3*** **Exons 7, 9 (alternative designation exon 10), 14, 18, Intron 17**	*FGFR4*	*FH*
FLCN	*FLT1*	***FLT3*** **Exons 14, 15, 20**	***FOXL2***	*FUBP1*	*GABRA6*	*GATA3*
GATA4	*GATA6*	***GNA11*** **Exons 4, 5**	*GNA13*	***GNAQ*** **Exons 4, 5**	***GNAS*** **Exons 1, 8**	*GRM3*
GSK3B	*H3F3A*	*HDAC1*	*HGF*	*HNF1A*	***HRAS*** **Exons 2, 3**	*HSD3B1*
ID3	***IDH1*** **Exon 4**	***IDH2*** **Exon 4**	*IGF1R*	*IKBKE*	*IKZF1*	*INPP4B*
IRF2	*IRF4*	*IRS2*	*JAK1*	***JAK2*** **Exon 14**	***JAK3*** **Exons 5, 11, 12, 13, 15, 16**	*JUN*
KDM5A	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	***KIT*** **Exons 8, 9, 11, 12, 13, 17,** Intron 16
KLHL6	*KMT2A (MLL)* Introns 6, 8-11, **Intron 7**	*KMT2D (MLL2)*	***KRAS***	*LTK*	*LYN*	*MAF*

Professional Service Gene List continued →

(Information Provided as a Professional Service Continued)

MAP2K1 (MEK1) **Exons 2, 3**	***MAP2K2 (MEK2)*** **Exons 2-4, 6, 7**	*MAP2K4*	*MAP3K1*	*MAP3K13*	*MAPK1*	*MCL1*
MDM2	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	***MET***
MITF	*MKNK1*	*MLH1*	***MPL*** **Exon 10**	*MRE11A*	*MSH2* Intron 5	*MSH3*
MSH6	*MST1R*	*MTAP*	***MTOR*** **Exons 19, 30, 39, 40, 43-45, 47, 48, 53, 56**	*MUTYHMYB** Intron 14	***MYC*** **Intron 1**	
MYCL (MYCL1)	***MYCN***	***MYD88*** **Exon 4**	*NBN*	*NF1*	*NF2*	*NFE2L2*
NFKBIA	*NKX2-1*	*NOTCH1*	*NOTCH2* Intron 26	*NOTCH3*	***NPM1*** **Exons 4-6, 8, 10**	***NRAS*** **Exons 2, 3**
NSD3 (WHSC1L1)	*NT5C2*	***NTRK1*** **Exons 14, 15, Introns 8-11**	*NTRK2* Intron 12	***NTRK3*** **Exons 16, 17**	*NUTM1** Intron 1	*P2RY8*
PALB2	*PARK2*	*PARP1*	*PARP2*	*PARP3*	*PAX5*	*PBRM1*
PDCD1 (PD-1)	***PDCD1LG2 (PD-L2)***	***PDGFRA*** **Exons 12, 18, Introns 7, 9, 11**	***PDGFRB*** **Exons 12-21, 23**	*PDK1*	*PIK3C2B*	*PIK3C2G*
PIK3CA **Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)**	*PIK3CB*	*PIK3R1*	*PIM1*	*PMS2*	*POLD1*	*POLE*
PPARG	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*	*PRKCI*	*PTCH1*
PTEN	***PTPN11***	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*
RAD51B	*RAD51C*	*RAD51D*	*RAD52*	*RAD54L*	***RAF1*** **Exons 3, 4, 6, 7, 10, 14, 15, 17,** Introns 4-8	*RARA* Intron 2
RB1	*RBM10*	*REL*	*RET* Introns 7, 8, **Exons 11, 13-16, Introns 9-11**	*RICTOR*	*RNF43*	***ROS1*** **Exons 31, 36-38, 40, Introns 31-35**
RPTOR	*RSPO2** Intron 1	*SDC4** Intron 2	*SDHA*	*SDHB*	*SDHC*	*SDHD*
SETD2	*SF3B1*	*SGK1*	*SLC34A2** Intron 4	*SMAD2*	*SMAD4*	*SMARCA4*
SMARCB1	***SMO***	*SNCAIP*	*SOCS1*	*SOX2*	*SOX9*	*SPEN*
SPOP	*SRC*	*STAG2*	*STAT3*	***STK11***	*SUFU*	*SYK*
TBX3	*TEK*	*TERC** ncRNA	***TERT**** **Promoter**	*TET2*	*TGFBR2*	*TIPARP*
*TMPRSS2** Introns 1-3	*TNFAIP3*	*TNFRSF14*	***TP53***	*TSC1*	*TSC2*	*TYRO3*
U2AF1	***VEGFA***	*VHL*	*WHSC1*	*WT1*	*XPO1*	*XRCC2*
ZNF217	*ZNF703*					

‡ Current as of November 2020. Please visit foundationmedicine.com for the most up-to-date gene list.

References:

1. FoundationOne Liquid CDx Technical Information. For full label refer to www.F1LCDxLabel.com
2. Data on File, Foundation Medicine, Inc., 2020

FoundationOne®Liquid CDx is for prescription use only and is a qualitative next-generation sequencing based *in vitro* diagnostic test for advanced cancer patients with solid tumors. The test analyzes 324 genes utilizing circulating cell-free DNA and is FDA-approved to report short variants in 311 genes and as a companion diagnostic to identify patients who may benefit from treatment with specific therapies (listed in Table 1 of the Intended Use) in accordance with the approved therapeutic product labeling. Additional genomic findings may be reported and are not prescriptive or conclusive for labeled use of any specific therapeutic product. Use of the test does not guarantee a patient will be matched to a treatment. A negative result does not rule out the presence of an alteration. Patients who are negative for companion diagnostic mutations should be reflexed to tumor tissue testing and mutation status confirmed using an FDA-approved tumor tissue test, if feasible. For the complete label, including companion diagnostic indications and complete risk information, please visit www.F1LCDxLabel.com.

FOUNDATION MEDICINE®

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