xF+ Validation

The Tempus xF+ (version 4) assay is a next-generation sequencing (NGS) cell-free DNA liquid biopsy tumor profiling assay for identifying genomic alterations derived from solid tumors but circulating in the blood. The 523 gene panel includes single nucleotide variants (SNVs), insertions and deletions (indels), copy number gains (CNGs), and chromosomal rearrangements detected by hybrid capture NGS using custom designed IDT probes. The assay uses 20 to 50 ng of input DNA. At 50 ng of input DNA, the xF+ assay has 97.9% sensitivity for SNVs in the enhanced region with variant allele fraction (VAF) ≥ 0.20%. Using 30 ng of input DNA, the xF+ assay has, 96.8% sensitivity for indels in the enhanced region with VAF ≥ 0.20%, >99.9% sensitivity for CNGs if circulating tumor DNA (ctDNA) tumor fraction is ≥ 1%, and 98.3% sensitivity for rearrangements if ctDNA tumor fraction is ≥ 0.5%. The assay also reports on Blood Tumor Mutational Burden (bTMB), and Microsatellite Instability High (MSI-H) status, variants potentially associated with clonal hematopoiesis (CH) and HLA Class I Genotyping. Tempus xF+ is designed to help capture clinically relevant biomarkers for solid tumors.

CAP/CLIA validation of the Tempus xF+ panel at Tempus' Chicago, Illinois laboratory focused on the detection of actionable oncologic variants including resistance mutations in plasma. The assay requires two 8.5 mL Streck tubes of peripheral blood. Clinical sequencing is performed to >5000x and >1500x unique coverage for enhanced and non-enhanced regions, respectively. Performance specifications are listed in the table below. These results establish high sensitivity and specificity for the Tempus xF+ assay.

Not intended for:

- Hematologic malignancies
- Early stage (stage I/II) cancers
- Primary CNS malignancies

xF+ PERFORMANCE SPECIFICATIONS

Variant Type	LOD	Sensitivity	Specificity	Lower Limit of Reporting*
SNVs (Enhanced)	0.20%	97.9%	>99.9%	≥0.05%
SNVs (Non Enhanced)	0.78%	95.8%	>99.9%	≥0.05%
INDELs (Enhanced)	0.20%	96.8%	>99.9%	≥0.05%
INDELs (Non Enhanced)	0.72%	>99.9%	>99.9%	≥0.05%
CNGs	1%	>99.9%	>99.9%	—
Rearrangements	0.5%	98.3%	>99.9%	—
MSI-H Status	—	—	>99.9%	≥16 unstable loci
bTMB	—	—	>99.9%	≥0.25% ctDNA TF

Performance characteristics for SNVs (Enhanced) established at 50 ng of DNA mass input. All other characteristics established at 30 ng of DNA mass input.

** In a select category of highly clinically relevant variants after pathologist review.*

xF+ Gene Panel

ABCC3 [2]	*BIRC3* [2]	*CREBBP* [2]	*ERBB4* [2]	*FLT1* [2]	*ID3* [2]	*KRAS* [1,4]	*MYB* [2]	*PHLPP2* [2]	*RAD51C* [1]	*SMARCB1* [2]	*TSC1* [1]
ABL1 [1,2]	*BLM* [2]	*CRKL* [1]	*ERCC2* [2]	*FLT3* [1]	*IDH1* [1]	*LATS1* [2]	*MYC* [1,4]	*PIAS4* [2]	*RAD51D* [2]	*SMC1A* [2]	*TSC2* [1]
ABL2 [2]	*BMPR1A* [2]	*CSF1R* [2]	*ERCC3* [2]	*FLT4* [2]	*IDH2* [1]	*LCK* [2]	*MYCL* [2]	*PIK3C2B* [2]	*RAD52* [2]	*SMC3* [2]	*TSHR* [2]
ABRAXAS1 [2]	*BRAF* [1,3,4]	*CSF3R* [2]	*ERCC4* [2]	*FOLH1* [2]	*IFNA21* [2]	*LMO1* [2]	*MYCN* [1]	*PIK3C2G* [2]	*RAD54L* [2]	*SMO* [1]	*TYMS* [2]
ACVR1 [2]	*BRCA1* [1]	*CTC1* [2]	*ERCC6* [2]	*FOXA1* [2]	*IFNAR1* [2]	*LRP1B* [2]	*MYD88* [1,2]	*PIK3CA* [1,4]	*RAF1* [1]	*SNCAIP* [2]	*TYRO3* [2]
ACVR1B [2]	*BRCA2* [1]	*CTCF* [2]	*ERG* [2]	*FOXL2* [1]	*IFNAR2* [2]	*LTK* [2]	*NBN* [2]	*PIK3CB* [2]	*RARA* [2]	*SOCS1* [2]	*U2AF1* [2]
AJUBA [2]	*BRD4* [2]	*CTLA4* [2]	*ERRFI1* [1]	*FOXO1* [2]	*IFNG* [2]	*LYN* [2]	*NCOA2* [2]	*PIK3CD* [2]	*RASA1* [2]	*SOS1* [2]	*UGT1A1* [2]
AKT1 [1]	*BRIP1* [2]	*CTNNA1* [2]	*ESR1* [1]	*FOXO3* [2]	*IFNGR1* [2]	*LZTR1* [2]	*NCOR1* [2]	*PIK3CG* [2]	*RB1* [1]	*SOX2* [2]	*VEGFA* [1]
AKT2 [1]	*BTG1* [2]	*CTNNB1* [1]	*ETNK1* [2]	*FOXP1* [2]	*IFNGR2* [2]	*MAF* [2]	*NF1* [1]	*PIK3R1* [1]	*RBM10* [2]	*SOX9* [2]	*VHL* [1]
AKT3 [2]	*BTG2* [2]	*CUL3* [2]	*ETV1* [2]	*FRS2* [2]	*IFNW1* [2]	*MALT1* [2]	*NF2* [1]	*PIK3R2* [2]	*RECQL4* [2]	*SPEN* [2]	*VSIR* [2]
ALK [1,3]	*BTK* [1]	*CUL4A* [2]	*ETV4* [2]	*FUBP1* [2]	*IGF1* [2]	*MAP2K1* [1]	*NFE2L2* [1]	*PIM1* [2]	*REL* [2]	*SPOP* [1]	*WEE1* [2]
ALOX12B [2]	*CALR* [2]	*CUX1* [2]	*ETV5* [2]	*GABRA6* [2]	*IGF1R* [2]	*MAP2K2* [1]	*NFKBIA* [2]	*PLCG1* [2]	*RET* [1,3,4]	*SRC* [2]	*WNK1* [2]
AMER1 [2]	*CARD11* [2]	*CXCR4* [2]	*ETV6* [2]	*GALNT12* [2]	*IKBKE* [2]	*MAP2K4* [2]	*NKX2-1* [2]	*PLCG2* [2]	*RHEB* [1]	*SRSF2* [2]	*WRN* [2]
APC [1]	*CARM1* [2]	*CYLD* [2]	*EWSR1* [2]	*GATA1* [2]	*IKZF1* [2]	*MAP3K1* [2]	*NOTCH1* [1]	*PMS1* [2]	*RHOA* [1]	*STAG2* [2]	*WT1* [2]
APLNR [2]	*CASP8* [2]	*CYP17A1* [2]	*EZH2* [1]	*GATA3* [1]	*IL10RA* [2]	*MAP3K13* [2]	*NOTCH2* [2]	*PMS2* [1]	*RICTOR* [2]	*STAT3* [2]	*XBP1* [2]
AR [1,4]	*CBFB* [2]	*CYSLTR2* [2]	*EZR* [2]	*GATA4* [2]	*IL32* [2]	*MAP3K21* [2]	*NOTCH3* [2]	*POLA1* [2]	*RIT1* [1]	*STAT5B* [2]	*XPA* [2]
ARAF [1]	*CBL* [2]	*DAXX* [2]	*FAM46C* [2]	*GATA6* [2]	*IL6R* [2]	*MAP3K7* [2]	*NOTCH4* [2]	*POLD1* [2]	*RNF43* [1]	*STAT6* [2]	*XPC* [2]
ARFRP1 [2]	*CCND1* [1]	*DDB2* [2]	*FANCA* [2]	*GID4* [2]	*IL7R* [2]	*MAPK1* [1]	*NPM1* [1]	*POLE* [2]	*ROS1* [1,3]	*STK11* [1]	*XPO1* [2]
ARID1A [1]	*CCND2* [1]	*DDR1* [2]	*FANCC* [2]	*GLI2* [2]	*IMPDH1* [2]	*MAPK3* [1]	*NQO1* [2]	*POLQ* [2]	*RPS6KB1* [2]	*SUFU* [2]	*XRCC1* [2]
ARID1B [2]	*CCND3* [1]	*DDR2* [1]	*FANCD2* [2]	*GNA11* [1]	*ING1* [2]	*MAX* [2]	*NRAS* [1]	*POT1* [2]	*RPTOR* [2]	*SUZ12* [2]	*XRCC2* [2]
ARID2 [2]	*CCNE1* [1,4]	*DDX3X* [2]	*FANCE* [2]	*GNA13* [2]	*INPP4B* [2]	*MC1R* [2]	*NRG1* [2,3]	*PPARG* [2]	*RRM1* [2]	*SYK* [2]	*YEATS4* [2]
ASNS [2]	*CD22* [2]	*DDX41* [2]	*FANCG* [2]	*GNAQ* [1]	*INSR* [2]	*MCL1* [2]	*NSD1* [2]	*PPM1D* [2]	*RSF1* [2]	*TBX3* [2]	*ZFHX3* [2]
ASXL1 [2]	*CD274(PD-L1)* [1,4]	*DEPTOR* [2]	*FANCI* [2]	*GNAS* [1]	*IRF1* [2]	*MDM2* [1,4]	*NSD2* [2]	*PPP2R1A* [2]	*RSPO2* [2]	*TCF7L2* [2]	*ZMYM3* [2]
ATM [1]	*CD70* [2]	*DICER1* [2]	*FANCL* [2]	*GPC3* [2]	*IRF2* [2]	*MDM4* [2]	*NSD3* [2]	*PPP2R2A* [2]	*RUNX1* [2]	*TEK* [2]	*ZNF217* [2]
ATR [1]	*CD74* [2]	*DIS3* [2]	*FANCM* [2]	*GPS2* [2]	*IRF4* [2]	*MED12* [2]	*NT5C2* [2]	*PPP6C* [2]	*RXRA* [2]	*TERC* [2]	*ZNF703* [2]
ATRX [2]	*CD79A* [2]	*DNMT1* [2]	*FAS* [2]	*GREM1* [2]	*IRS2* [2]	*MEF2B* [2]	*NTRK1* [1,3]	*PRDM1* [2]	*SDC4* [2]	*TERT* [1]	*ZNF750* [2]
AURKA [2]	*CD79B* [2]	*DNMT3A* [2]	*FAT1* [2]	*GRIN2A* [2]	*JAK1* [1]	*MEN1* [2]	*NTRK2* [1,2,3]	*PREX2* [2]	*SDHA* [1]	*TET2* [2]	*ZNRF3* [2]
AURKB [2]	*CDC73* [2]	*DOT1L* [2]	*FBXW7* [1]	*GRM3* [2]	*JAK2* [1]	*MERTK* [2]	*NTRK3* [1,3]	*PRKACA* [2]	*SDHAF2* [2]	*TFEB* [2]	*ZRSR2* [2]
AURKC [2]	*CDH1* [1]	*DPYD* [2]	*FCGR2A* [2]	*GSK3B* [2]	*JAK3* [1]	*MET* [1,4]	*NUTM1* [2]	*PRKAR1A* [2]	*SDHB* [2]	*TGFB1* [2]	
AXIN1 [2]	*CDK12* [1]	*EBF1* [2]	*FCGR3A* [2]	*GSTP1* [2]	*JUN* [2]	*MITF* [2]	*P2RY8* [2]	*PRKCI* [2]	*SDHC* [2]	*TGFBR1* [2]	
AXIN2 [2]	*CDK4* [1]	*EED* [2]	*FGF10* [2]	*H3F3A* [2]	*KAT6A* [2]	*MKNK1* [2]	*PAK1* [2]	*PRKN* [2]	*SDHD* [2]	*TGFBR2* [2]	
AXL [2]	*CDK6* [1]	*EEF2* [2]	*FGF12* [2]	*HAVCR2* [2]	*KDM5A* [2]	*MLH1* [1]	*PALB2* [1]	*PTCH1* [1]	*SETBP1* [2]	*TIGIT* [2]	
B2M [1]	*CDK8* [2]	*EGFR* [1,4]	*FGF14* [2]	*HDAC1* [2]	*KDM5C* [2]	*MLH3* [2]	*PALLD* [2]	*PTEN* [1]	*SETD2* [2]	*TIPARP* [2]	
BAP1 [1]	*CDK9* [2]	*EGLN1* [2]	*FGF19* [2]	*HDAC2* [2]	*KDM5D* [2]	*MPL* [1]	*PARP1* [2]	*PTK2* [2]	*SF3B1* [2]	*TMEM127* [2]	
BARD1 [2]	*CDKN1A* [2]	*EIF1AX* [2]	*FGF23* [2]	*HGF* [2]	*KDM6A* [2]	*MRE11* [2]	*PARP2* [2]	*PTPN11* [1]	*SGK1* [2]	*TMPRSS2* [2]	
BAX [2]	*CDKN1B* [2]	*ELF3* [2]	*FGF3* [2]	*HIF1A* [2]	*KDR* [1]	*MS4A1* [2]	*PARP3* [2]	*PTPN13* [2]	*SIRPA* [2]	*TNFAIP3* [2]	
BCL2 [2]	*CDKN2A* [1]	*EMSY* [2]	*FGF4* [2]	*HIST1H3B* [2]	*KEAP1* [1]	*MSH2* [1]	*PAX5* [2]	*PTPRD* [2]	*SLC34A2* [2]	*TNFRSF14* [2]	
BCL2L1 [2]	*CDKN2B* [2]	*EP300* [2]	*FGF6* [2]	*HLA-B* [5]	*KEL* [2]	*MSH3* [1]	*PBRM1* [1]	*PTPRO* [2]	*SLC9A3R1* [2]	*TNFRSF17* [2]	
BCL2L11 [2]	*CDKN2C* [2]	*EPCAM* [2]	*FGFR1* [1,3]	*HNF1A* [1]	*KIT* [1,4]	*MSH6* [1]	*PDCD1* [2]	*PTPRT* [2]	*SLFN11* [2]	*TOP1* [2]	
BCL2L2 [2]	*CEBPA* [2]	*EPHA2* [2]	*FGFR2* [1,3]	*HNF1B* [2]	*KLF4* [2]	*MST1R* [2]	*PDCD1LG2* [1]	*QKI* [2]	*SLIT2* [2]	*TOP2A* [2]	
BCL6 [2]	*CHD4* [2]	*EPHA3* [2]	*FGFR3* [1,3]	*HOXB13* [2]	*KLHL6* [2]	*MTAP* [2]	*PDGFRA* [1]	*RAC1* [2]	*SMAD2* [2]	*TP53* [1]	
BCLAF1 [2]	*CHEK1* [2]	*EPHB1* [2]	*FGFR4* [1]	*HRAS* [1]	*KLLN* [2]	*MTHFR* [2]	*PDGFRB* [1]	*RAD21* [2]	*SMAD3* [2]	*TP53BP1* [2]	
BCOR [2]	*CHEK2* [1]	*EPHB4* [2]	*FH* [2]	*HSD3B1* [2]	*KMT2A* [1]	*MTOR* [1]	*PDK1* [2]	*RAD50* [2]	*SMAD4* [1]	*TP63* [2]	
BCORL1 [2]	*CIC* [2]	*ERBB2(HER2)* [1,4]	*FHIT* [2]	*HSP90AA1* [2]	*KMT2C* [2]	*MUC16* [2]	*PHGDH* [2]	*RAD51* [2]	*SMARCA2* [2]	*TRAF3* [2]	
BCR [2]	*CKS1B* [2]	*ERBB3* [1,2]	*FLCN* [2]	*HSPH1* [2]	*KMT2D* [2]	*MUTYH* [2]	*PHLPP1* [2]	*RAD51B* [2]	*SMARCA4* [2]	*TRAF7* [2]	

GENE REARRANGEMENTS

*ALK, BRAF, FGFR1, FGFR2, FGFR3, NRG1**, NTRK1, NTRK2, NTRK3, RET, ROS1*

COPY NUMBER GAINS

AR, BRAF, CCNE1, CD274 (PD-L1), EGFR, ERBB2 (HER2), KIT, KRAS, MDM2, MET, MYC, PIK3CA, RET

*** Detection of NRG1 fusions is largely incidental due to select intronic and non-enhanced coverage.*

1 *Enhanced SNV/Indel* 2 *Non-Enhanced SNV/Indel* 3 *Fusion* 4 *CNV* 5 *HLA Genotyping*