

xT Validation

The Tempus xT next generation sequencing assay is designed to detect actionable oncologic targets by sequencing tumor samples with matched normal saliva or blood samples, when available.

The xT assay¹ covers 648 genes spanning ~3.6 Mb of genomic space. From DNA sequencing, somatic and incidentally detected germline single nucleotide variants (SNVs), insertions and deletions (indels), copy number variants (CNVs), and translocations in 22 genes are detected, along with the TERT promoter region, and 239 sites to determine microsatellite instability status. Tumor mutational burden (TMB) and microsatellite instability (MSI) status are reported. HLA Class I genotyping information is provided on xT tumor normal and tumor only reports for clinical trial matching purposes only, and not for transplantation purposes. Test results are intended to provide tumor molecular information that can be used by clinicians to help inform clinical management when patients are seeking further cancer treatment.

CAP/CLIA validation of the Tempus xT panel in its Chicago, Illinois and Durham, North Carolina laboratories focused on actionable oncologic variants. The assay requires specimens with a tumor content of 20% post macrodissection (minimum 30% for MSI status). For solid tumors, an FFPE tumor sample is sequenced along with a matched normal blood or saliva sample (when available). For circulating hematologic malignancies, a blood or bone marrow sample is sequenced. Clinical sequencing is performed to 500x depth of coverage for tumor specimens and 150x for normal specimens. Performance specifications are listed in Tables 1 and 2 below. These results establish high sensitivity and specificity for the Tempus xT assay. The xT assay is used across a diverse set of clinical settings including leading academic centers, NCI designated cancer centers, hospital networks, and community hospitals.

xT PERFORMANCE SPECIFICATIONS—CHICAGO LAB*

Variant Class	Limit of Detection	Analytical Sensitivity	Negative Percentage Agreement
SNVs	5.0% VAF	98.2%	>99.0%
Indels	5.0% VAF	91.1%	>99.0%
Copy Number Alterations	Gain: 30.0% Tumor Purity Loss: 40.0% Tumor Purity	91.4%	>99.0%
Microsatellite Instability	30.0% Tumor Purity	90.5%	98.4%
Rearrangements	30.0% Tumor Purity	90.9%	>99.0%

xT PERFORMANCE SPECIFICATIONS—DURHAM LAB*

Variant Class	Limit of Detection	Analytical Sensitivity	Negative Percentage Agreement
SNVs	5.0% VAF	97.6%	>99.0%
Indels	5.0% VAF	91.4%	>99.0%
Copy Number Alterations	Gain: 33.2% Tumor Purity Loss: 38.5% Tumor Purity	92.7%	>99.0%
Microsatellite Instability	30.0% Tumor Purity	96.2%	99.6%
Rearrangements	30.0% Tumor Purity	96.3%	>99.0%

¹ Version 4 of the Tempus xT assay. * Solid tumor performance

xT Gene List

ABC11	BCR	CHD2	(TCEB1)	FCGR3A	GRIN2A	IFNAR1	MAD2L2	NFKBIA	PIK3R1	RHOA	SPOP	TSHR
ABCC3	BIRC3	CHD4	EMSY	FDPS	GRM3	IFNAR2	MAF	NHP2	PIK3R2 †	RICTOR	SPRED1	TUSC3
ABL1	BLM ***	CHD7	ENG	FGF1	GSTP1	IFNGR1	MAFB	NKX2-1	PIM1	RINT1	SRC	TYMS
ABL2	BMP1R1 ***	CHEK1	EP300	FGF10	H19	IFNGR2	MAGI2	NOX1	PLCG1	RIT1	SRSF2	U2AF1
ABRAXAS1	BRAF	CHEK2 ***	EPCAM *** †	FGF14	H3F3A	IFNL3	MALT1	NOTCH1	PLCG2	RNF139	STAG2	UBE2T
ACTA2	BRCA1 ***	CIC	EPHA2	FGF2	HAS3	IKBKE	MAP2K1	NOTCH2	PML	RNF43	STAT3	UGT1A1
ACVR1 (ALK2)	BRCA2 ***	CIITA	EPHA7	FGF23	HAVCR2	IKZF1	MAP2K2	NOTCH3	PMS1	ROS1	STAT4	UGT1A9
ACVR1B	BRD4	CKS1B	EPHB1	FGF3	HDAC1	IL10RA	MAP2K4	NOTCH4	PMS2 ***	RPL5	STAT5A	UMPS
AGO1	BRIP1 ***	CREBBP	EPHB2	FGF4	HDAC2	IL15	MAP3K1	NPM1	POLD1 ***	RPS15	STAT5B	VEGFA
AJUBA	BTG1	CRKL	EPOR	FGF5	HDAC4	IL2RA	MAP3K7	NQO1	POLE ***	RPS6KB1	STAT6	VEGFB
AKT1	BTK	CRLF2	ERBB2 (HER2)	FGF6	HGF	IL6R	MAPK1	NRAS	POLH	RPTOR	STK11 ***	VHL ***
AKT2	BUB1B	CSF1R	ERBB3	FGF7	HIF1A	IL7R	MAX ***	NRG1	POLQ	RRM1	SUFU ***	VSIR
AKT3	C11orf65	CSF3R	ERBB4	FGF8	HIST1H1E	ING1	MC1R	NSD1	POT1	RSF1	SUZ12	WEE1
ALK	C3orf70	CTC1	ERCC1	FGF9	HIST1H3B	INPP4B	MCL1	NSD2	POU2F2	RUNX1 ***	SYK	WNK1
AMER1	C8orf34	CTCF	ERCC2	FGFR1	HIST1H4E	IRF1	MDM2	NT5C2	PPARA	RUNX1T1	SYNE1	WNK2
APC ***	CALR	CTLA4	ERCC3	FGFR2	HLA-A	IRF2	MDM4	NTHL1 ***	PPARD	RXRA	TAF1	WRN
APLN1	CARD11	CTNNA1	ERCC4	FGFR3	HLA-B	IRF4	MED12	NTRK1	PPARG	SCG5	TANC1	WT1 ***
APOB	CARM1	CTNNA1	ERCC5	FGFR4	HLA-C	IRS2	MEF2B	NTRK2	PPM1D	SDHA ***	TAP1	XPA
AR	CASP8	CTRC	ERCC6	FH ***	HLA-DMA	ITPKB	MEN1 ***	NTRK3	PPP1R15A	SDHAF2 ***	TAP2	XPC
ARAF	CASR	CUL1	ERG	FHIT	HLA-DMB	JAK1	MET ***	NUDT15	PPP2R1A	SDHB ***	TARBP2	XPO1
ARHGAP26	CBFB	CUL3	ERRFI1	FLCN ***	HLA-DOA	JAK2	MGMT	NUP98	PPP2R2A	SDHC ***	TBC1D12	XRCC1
ARHGAP35	CBL	CUL4A	ESR1	FLT1	HLA-DOB	JAK3	MIB1	OLIG2	PPP6C	SDHD ***	TBL1XR1	XRCC2
ARID1A	CBLB	CUL4B	ETS1	FLT3	HLA-DPA1	JUN	MITF	P2RY8	PRCC	SEC23B	TBX3	XRCC3
ARID1B	CBLC	CUX1	ETS2	FLT4	HLA-DPB1	KAT6A	MKI67	PAK1	PRDM1	SEMA3C	TCF3	YEATS4
ARID2	CBR3	CXCR4	ETV1	FNTB	HLA-DPB2	KDM5A	MLH1 ***	PALB2 ***	PREX2	SETBP1	TCF7L2	ZFH3
ARID5B	CCDC6	CYLD	ETV4	FOXA1	HLA-DQA1	KDM5C	MLH3	PALLD	PRKAR1A ***	SETD2	TCL1A	ZMYM3
ASNS	CCND1	CYP11B1	ETV5	FOXL2	HLA-DQA2	KDM5D	MLL2	NUP98	PRKDC	SF3B1	TERT **	ZNF217
ASPSR1	CCND2	CYP2D6	ETV6 ***	FOXO1	HLA-DQB1	KDM6A	MN1	OLIG2	PRKDC	SGK1	TET2	ZNF471
ASXL1	CCND3	CYP3A5	ETS1	FOXO3	HLA-DQB2	KDR	MPL	P2RY8	PRKN	SH2B3	TFE3 †	ZNF620
ATIC	CCNE1	CYSLTR2	ETS2	FOXP1	HLA-DRA	KEAP1	MRE11	PAK1	PRSS1	SHH	TFEB	ZNF750
ATM ***	CD19	DAXX	ETV1	FOXQ1	HLA-DRB1	KEL	MS4A1	PAX3	PTCH1 ***	SLC26A3	TFEC	ZNRF3
ATP7B	CD22	DDB2	ETV4	FRS2	HLA-DRB5	KIF1B	MSH2 ***	PAX5	PTCH2	SLC47A2	TGFBF1	ZRSR2
ATR	CD274 (PD L1)	DDR2	ETV5	FUBP1	HLA-DRB6	KIT ***	MSH3 ***	PAX7	PTEN ***	SLC9A3R1	TGFBF2	
ATRX	CD40	DDX3X	ETV6 ***	FUS	HLA-E	KLF4	MSH6 ***	PAX8	PTPN11	SLIT2	TIGIT	
AURKA	CD70	DICER1 ***	EWSR1	G6PD	HLA-F	KLHL6	MTAP	PBRM1	PTPN13	SLX4	TMEM127 ***	
AURKB	CD79A	DIRC2	EZH2	GABRA6	HLA-G	KLLN	MTHFD2	PCBP1	PTPN22	SMAD2	TMEM173	
AXIN1	CD79B	DIS3	FAM46C	GALNT12	HNFB1	KMT2A	MTHFR	PDCD1 †	PTPRD	SMAD3	TMPRSS2	
AXIN2 ***	CDC73	DIS3L2	FANCA	GATA1	HNFB1	KMT2B	MTOR	PDCD1LG2	PTPRT	SMAD4 ***	TNF	
AXL	CDH1 ***	DKC1	FANCB	GATA2 ***	HOXA11	KMT2C	MTRR	PDGFRA ***	RAD51	SMARCA1 †	TNFAIP3	
B2M	CDK12	DNM2	FANCC	GATA3	HOXB13	KMT2D	MUTYH ***	PDGFRB	RAD51B	SMARCA4 ***	TNFRSF14	
BAP1 ***	CDK4 ***	DNMT3A	FANCD2	GATA4	HRAS	KRAS	MYB	PDK1	RAD51C ***	SMARCB1 ***	TNFRSF17	
BARD1 ***	CDK6	DOT1L	FANCE	GATA6	HSD11B2	L2HGDH	MYC	PHF6	RAD51D ***	SMARCE1	TNFRSF9	
BCL10	CDK8	DPYD	FANCF	GEN1	HSD3B1	LAG3	MYCL	PHGDH	RAD54L	SMC1A	TOP1	
BCL11B	CDKN1A	DYNC2H1	FANCG	GLI1	HSD3B2	LATS1	MYCN	PHLPP1	RAF1	SMC3	TOP2A	
BCL2	CDKN1B	EBF1	FANCI	GLI2	HSP90AA1	LCK	MYD88	PHLPP2	RANBP2	SMO	TP53 ***	
BCL2L1	CDKN1C	ECT2L	FANCL	GNA11	HSPH1	LDLR	MYH11	PHOX2B ***	RARA	SOCS1	TP63	
BCL2L11	CDKN2A ***	EGF	FANCM	GNA13	IDH1	LEF1	NBN ***	PIAS4	RASA1	SOD2	TPM1	
BCL6	CDKN2B	EGFR ***	FAS	GNAQ	IDH2	LMNA	NCOR1	PIK3C2B	RB1 ***	SOX10	TPMT	
BCL7A	CDKN2C	EGLN1	FAT1	GNAS	IDO1	LMO1	NCOR2	PIK3CA	RBM10 †	SOX2	TRAF3	
BCLAF1 †	CEBPA ***	EIF1AX	FBXO11	GPC3	IFIT1	LRP1B	NF1 ***	PIK3CB	RECQL4	SOX9	TRAF7	
BCOR	CEP57	ELF3	FBXW7	GPS2	IFIT2	LYN	NF2 ***	PIK3CD	RET ***	SPEN	TSC1 ***	
BCORL1	CFTF	ELOC	FCGR2A	GREM1	IFIT3	LZTR1	NFE2L2	PIK3CG	RHEB	SPINK1	TSC2 ***	

GENE REARRANGEMENTS BY DNA SEQUENCING †

ABL1, ALK, BCR, BRAF, EGFR, ETV6, EWSR1, FGFR2, FGFR3, MYB, NRG1, NTRK1, NTRK2, NTRK3, PAX8, PDGFRA, PML, RARA, RET, ROS1, TFE3, TMPRSS2

2 NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic V.2.2022. © National Comprehensive Cancer Network, Inc. 2022. All rights reserved. Accessed [July 19, 2022]. To view the most recent and complete version of the guideline, go online to NCCN.org.

3 NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Colorectal V.1.2022. © National Comprehensive Cancer Network, Inc. 2022. All rights reserved. Accessed [July 19, 2022]. To view the most recent and complete version of the guideline, go online to NCCN.org.

** Includes promoter region

*** Genes in which potential germline findings are reported

In addition to reporting on somatic variants, when a normal sample is provided, Tempus reports germline potential findings on a limited set of variants associated with inherited cancer syndromes within 65 genes selected based on recommendations from the American College of Medical Genetics (ACMG), the National Comprehensive Cancer Network (NCCN), and/or published literature.

† Exons and select intronic regions only. Additional coverage information provided upon request.

‡ Genes that do not have full exon coverage of a primary transcript

Potential germline findings gene list

This list is composed of 65 genes associated with inherited cancer syndromes included on the xT panel and selected based on recommendations from the American College of Medical Genetics (ACMG), the National Comprehensive Cancer Network (NCCN) and/or published literature.^{2,3} The primary focus of the xT panel is somatic reporting. Tempus also offers separately ordered validated germline hereditary cancer panels through two reference labs, Ambry Genetics® and GeneDx, for xG testing, with the choice of reference lab depending on various factors. As each test differs, please contact your Tempus representative for more details on the variances between GeneDx and Ambry testing.

APC: APC-associated conditions

ATM: Ataxia-Telangiectasia, Breast cancer susceptibility, Pancreatic cancer susceptibility

AXIN2: Oligodontia-colorectal cancer syndrome

BAP1: BAP1 tumor predisposition syndrome

BAR1: Breast cancer susceptibility

BLM: Bloom syndrome

BMPR1A: Juvenile polyposis

BRCA1: Hereditary breast and ovarian cancer

BRCA2: Hereditary breast and ovarian cancer, Fanconi anemia

BRIP1: Ovarian cancer susceptibility, Fanconi anemia

CDH1: Hereditary diffuse gastric cancer, Breast cancer susceptibility

CDK4: Melanoma susceptibility

CDKN2A: Melanoma-pancreatic cancer syndrome

CEBPA: Acute myeloid leukemia susceptibility

CHEK2: Breast cancer susceptibility, Colon cancer susceptibility

DICER1: DICER1 tumor predisposition syndrome

EGFR §: Lung cancer susceptibility, TKI resistance

EPCAM §: Lynch syndrome

ETV6: Leukemia susceptibility, thrombocytopenia susceptibility

FH: Hereditary leiomyomatosis and renal cell cancer

FLCN: Birt-Hogg-Dube syndrome

GATA2: GATA2 deficiency with susceptibility to myeloid malignancies

KIT: Familial gastrointestinal stromal tumor

MAX: Hereditary paraganglioma-pheochromocytoma syndrome

MEN1: Multiple endocrine neoplasia type 1

MET: Hereditary papillary renal cell carcinoma

MLH1: Lynch syndrome, Constitutional mismatch repair deficiency

MSH2: Lynch syndrome, Constitutional mismatch repair deficiency

MSH3: MSH3-associated polyposis

MSH6: Lynch syndrome, Constitutional mismatch repair deficiency

MUTYH: MUTYH-associated polyposis

NBN: Nijmegen breakage syndrome, Breast cancer susceptibility

NF1: Neurofibromatosis type 1

NF2: Neurofibromatosis type 2

NTHL1: NTHL1 tumor syndrome, NTHL1-associated polyposis

PALB2: Breast cancer susceptibility, Pancreatic cancer susceptibility, Ovarian cancer susceptibility, Fanconi anemia

PDGFRA: Familial gastrointestinal stromal tumor, GIST-plus syndrome

PHOX2B: Neuroblastoma susceptibility

PMS2: Lynch syndrome, Constitutional mismatch repair deficiency

POLD1 §: Polymerase proofreading-associated polyposis

POLE §: Polymerase proofreading-associated polyposis

PRKAR1A: Carney complex

PTCH1: Gorlin syndrome, Basal cell nevus syndrome

PTEN: PTEN hamartoma tumor syndrome

RAD51C: Ovarian cancer susceptibility, Breast cancer susceptibility, Fanconi anemia

RAD51D: Ovarian cancer susceptibility, Breast cancer susceptibility

RB1: Retinoblastoma

RET: Multiple endocrine neoplasia type 2, Familial medullary thyroid cancer

RUNX1: Acute myeloid leukemia susceptibility

SDHA: Hereditary paraganglioma-pheochromocytoma syndrome

SDHAF2: Hereditary paraganglioma-pheochromocytoma syndrome

SDHB: Hereditary paraganglioma-pheochromocytoma syndrome

SDHC: Hereditary paraganglioma-pheochromocytoma syndrome

SDHD: Hereditary paraganglioma-pheochromocytoma syndrome

SMAD4: Juvenile polyposis, Hereditary hemorrhagic telangiectasia

SMARCA4: Rhabdoid tumor predisposition syndrome

SMARCB1: Rhabdoid tumor predisposition syndrome, Schwannomatosis

STK11: Peutz-Jeghers syndrome

SUFU: Gorlin syndrome, Basal cell nevus syndrome

TMEM127: Hereditary paraganglioma-pheochromocytoma syndrome

TP53: Li-Fraumeni syndrome

TSC1: Tuberous sclerosis complex

TSC2: Tuberous sclerosis complex

VHL: Von Hippel-Lindau syndrome

WT1: WT1-related Wilms tumor

§ Special reporting – EGFR: p.T790M, p.L792H, p.C797G, p.C797S (resistance alterations only); EPCAM: Large deletions only; POLD1: Exonuclease domain only; POLE: Exonuclease domain only.