

Precise™ Tumor

Molecular Profile Test

A pan-cancer solid tumor comprehensive genomic profiling test

Precise™ Tumor simplifies patient care for oncologists by providing straightforward interpretations, prioritization of therapies, and the next steps specific to each patient's genomic results.

Precise Tumor is a comprehensive laboratory test offered by Myriad Genetics that uses state-of-the-art next-generation targeted exome sequencing to discover and target important variants within tumors. This hybrid capture DNA- and RNA-based test detects Single Nucleotide Variants (SNVs), Insertions/deletions (INDELs), Copy Number Variants (CNVs), splice variants and fusions in solid tumors. Sequencing of over 500 genes, identified as relevant to cancer treatment, as well as testing of important immunotherapy (IO) biomarkers provides a comprehensive picture for the oncologist to formulate a treatment plan with their patients.

Product Features:

- **Actionable:** 500+ solid tumor-related genes with broad coverage of key guidelines and clinical trials for multiple solid tumor types.
- **Key Immuno-oncology biomarkers:**
 - Microsatellite Instability (MSI)
 - Tumor Mutational Burden (TMB)
 - Programmed Death-Ligand 1 (PD-L1), if requested.
- **Clinically Validated and Comprehensive** genomic coverage necessary for accurate selection of FDA-approved targeted and IO therapies, while also evaluating many genes relevant to early phase drug development efforts that may have future clinical applications in all solid tumors¹.
- **Accurate:** Sensitivity: 98.91% Specificity >99.99% using orthogonal methods, including DNA- and RNA-based next generation targeted exome sequencing with full coverage of all exons. Accurately measures TMB, microsatellite instability, SNVs, indels, copy-number/structural variation and gene fusions when compared to WGS and orthogonal technologies².
- **Cutting edge technologies:** Fusions and splice variants assessed via RNA analysis, which may detect more actionable fusions and splice variants, as compared to DNA tests only³⁻⁶.

Precise Tumor detects clinically relevant DNA & RNA variants for multiple solid-tumor cancer types.

Some key cancer-specific biomarkers include:

Breast	Ovarian	Endometrial	Colon	Pancreatic	Prostate	Lung	Pan-Cancer	
ATK1 AR BRCA1 BRCA2 ERBB2	BRCA1 BRCA2 KRAS PDGFRA FOXL2 TP53	POLE MLH1 MSH2 PMS2 MSH6 TP53 ERBB2	AKT1 BRAF HRAS KRAS MET MLH1	ALK BRAF BRCA1 BRCA2 ERBB2 KRAS NRG1 PALB2 ROS1	ATM BRCA1 BRCA2 MLH1 MSH2 PMS2 MSH6 AR	AKT1 ALK BRAF DDR2 EGFR ERBB2 FGFR1 FGFR2 FGFR3	KRAS MAP2K1 MET NRAS PIK3CA PTEN RET TP53 PD-L1	NTRK1 NTRK2 NTRK3 TMB MSI

Assay Specifications:

Sample Requirements*	Cancer Type: Solid Tumor Specimen Types: Cytology Cell Block
FFPE block requirement*	Cross-sectional tumor area of 25mm ² containing at least 40 μm of tumor
Tumor Purity Minimum	20%
DNA input required	40 ng
RNA input required	40 ng
Limit of detection	5% VAF for SNV and INDELs 10 copies/ng for fusions 2.5-fold change for CNV
Panel size	523 genes assessed by DNA analysis 56 genes assessed by RNA analysis
Average total coverage/Depth of coverage	Average 825x (Validated average of >500x) Since launch, average DNA reads (80M, or 825x) has remained above the stated validated average. Similarly, the average RNA reads (25M, or 1818x) is also high.
Analytical Sensitivity	99.25%
Analytical Specificity	>99.9%

*See Precise Tumor Pathology Guide for additional details

Overview of Performance Characteristics (at ≥20% Tumor Nuclei):**

Variant Type	Sensitivity (Positive Percent Agreement- PPA) (95% CI)	Specificity (Negative Percent Agreement-NPA) (95% CI)
All Variants	98.91% (98.31-99.34%)	>99.99% (>99.99%)
SNV	99.42% (98.81-99.76%)	>99.99% (>99.99%)
INDEL (≤ 40bp)	97.22% (94.36-98.83%)	>99.99% (>99.99%)
CNV	97.98% (95.27-99.31%)	100% (99.52-100%)
Fusions	99.12% (95.94-99.90%)	100% (99.45-100%)
MET exon 14 skipping	100% (46.44-99.98%)	100% (73.78-99.99%)

Variant Type	Positive Predictive Value (PPV) (95% CI)	Negative Predictive Value (NPV) (95% CI)
All Variants	100% (99.95-100%)	>99.9% (>99.99%)
Single Nucleotide Variants (SNV)	100% (99.95%-100%)	>99.99% (>99.99%)
Insertions/Deletions (INDEL)	100% (97.69-100%)	>99.99% (>99.99%)
Copy Number Variants (CNV)	100% (66.96-99.99%)	100% (99.52-100%)
Fusions	100% (91.49-100%)	99.78% (99.45-100%)
MET exon 14 skipping	100% (46.44-99.98%)	100% (73.78-99.99%)

**PPV, PPA, NPA, NPV were determined to be acceptable for all variant types (≥90% for SNVs, CNVs, fusions, and MET exon 14 skipping; ≥85% for INDELs).

Positive Percent Agreement (PPA): The conditional probability that a variant will be correctly identified by a clinical test, i.e. the number of true positive results divided by the total number of variants identified by the test (which is the sum of the numbers of true positive plus false negative results).

Negative Percent Agreement (NPA): The conditional probability that a negative/wildtype variant will be correctly identified by a clinical test, i.e. the number of true negative results divided by the total number of those without the disease (which is the sum of the numbers of true negative plus false positive results).

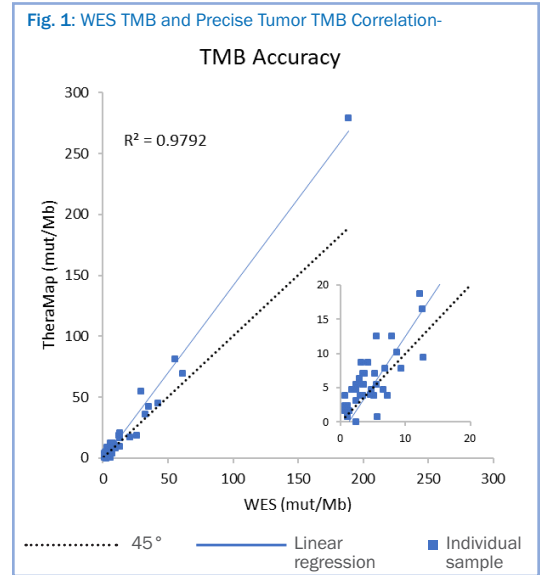
Positive Predictive Value (PPV): The conditional probability that positive/mutant variants will be correctly identified by a clinical test, i.e. the number of true positive results divided by the total number detected or classified as mutant (which is the sum of the numbers of true positive plus false positive results).

Negative Predictive Value (NPV): The conditional probability that the absence of a variant will be correctly identified by a clinical test, i.e. the number of true negative results divided by the total number without the disease (which is the sum of the numbers of true negatives plus false positives results).

Additional information about immuno-oncology biomarkers:

Tumor mutation burden (TMB):

Results are reported as low or high. A high TMB score has been shown to correlate with response to checkpoint inhibitor therapy and in some clinical contexts may indicate the use of Pembrolizumab or other immunotherapies. Following the FDA’s labeling for Pembrolizumab in solid tumors, a TMB value of 10 or greater is considered “High”, while values below 10 mut/Mb are considered “Low”. The cutoffs were determined across several tumor types in an internal validation study in comparison to whole exome sequencing. TMB Accuracy was measured by comparing the TMB values generated by Precise Tumor against reference TMB generated from paired tumor normal Whole Exome Sequencing (WES). For the sample set evaluated and presented in Fig. 1. TMB accuracy exceeded the acceptability criteria with an $R^2 = 0.9792$ (acceptability criteria $R^2 \geq 0.85$ between WES and Precise Tumor TMB values). This cutoff is supported by Wei et al. and the Friends of Cancer TMB Harmonization Project, which establishes 10 mut/Mb as a TMB-high cutoff in pembrolizumab monotherapy study-enrolled patients^{7,8,9}



Microsatellite instability (MSI):

The Precise Tumor assay evaluates 130 loci. Samples with >10% unstable sites are considered “MSI-High Positive”. The assay does not distinguish between “MS-Stable” and “MSI-Low” due to the limitations of tumor only MSI testing. MSI status may be reported as “Indeterminate” if the total number of usable sites is less than 55. The percentage of unstable microsatellites in 26 orthogonally confirmed microsatellite stable (MSS) or MSI negative samples were used to establish MSS cutoff of 10% unstable sites based on 3 standard deviations from the mean. A second cohort of orthogonally confirmed clinical specimens with MSS (tumor/normal) or MSI negative (tumor only) and MSI high (tumor/normal) or MSI Positive (tumor only) and 1 MSI positive cell-line were tested utilizing a 10% cutoff. PPA, PPV, and NPA were above the acceptability criteria of 90%. Based on limited availability and lack of clinical actionability, there is insufficient evidence to establish a low/intermediate range and MSI low samples were excluded from the analysis. MSI classification is reported as either positive or negative for MSI.

Variant Type	Sensitivity (PPA) (95% CI)	Positive Predictive Value (PPV) (95% CI)	Negative Percent Agreement (NPA) (95% CI)
MSI	100% (72.25-100%)	100% (78.28-100%)	100% (94.6-100%)

PD-L1:

Precise Tumor uses an immunohistochemical assay (SP263 clone) for the assessment of PD-L1 protein in solid tumor formalin-fixed paraffin-embedded (FFPE) tissue stained with OptiView DAB IHC Detection Kit. Results may be reported as either Tumor Proportion Score (TPS) or Combined Positive Score (CPS) depending on the cancer type. A TPS score of $\geq 1\%$ PD-L1 is reported as positive. A CPS score ≥ 1 or ≥ 10 expression is reported as positive.

Tumor Proportion Score (TPS)	Combined Positive Score (CPS)
All primary lung cancers ($\geq 1\%$)	Gastric and Gastroesophageal Adenocarcinomas (≥ 1)
Melanoma ($\geq 1\%$)	Head and Neck Squamous Cell Carcinomas (≥ 1)
All remaining solid tumors ($\geq 1\%$)	Cervical Carcinoma (≥ 1)
	Urothelial Carcinoma (≥ 10)
	Esophageal Squamous Cell Carcinoma (≥ 10)
	Triple negative breast cancer (≥ 10)

Table 1: PD-L1 positive expression ranges by cancer type.

Solid tumor panel

523 genes by DNA sequencing: • SNVs • Indels • CNVs

(Content shaded in grey is analyzed for CNV detection.)

ABL1	CALR	DNAJB1	FGF14	HIST1H3A	KEAP1	MYCL1	PIK3CD	RHOA	SUFU
ABL2	CARD11	DNMT1	FGF19	HIST1H3B	KEL	MYCN	PIK3CG	RICTOR	SUZ12
ACVR1	CASP8	DNMT3A	FGF2	HIST1H3C	KIF5B	MYD88	PIK3R1	RIT1	SYK
ACVR1B	CBFB	DNMT3B	FGF23	HIST1H3D	KIT	MYOD1	PIK3R2	RNF43	TAF1
AKT1	CBL	DOT1L	FGF3	HIST1H3E	KLF4	NAB2	PIK3R3	ROS1	TBX3
AKT2	CCND1	E2F3	FGF4	HIST1H3F	KLHL6	NBN	PIM1	RPS6KA4	TCEB1
AKT3	CCND2	EED	FGF5	HIST1H3G	KMT2B	NCOA3	PLCG2	RPS6KB1	TCF3
ALK	CCND3	EGFL7	FGF6	HIST1H3H	KMT2C	NCOR1	PLK2	RPS6KB2	TCF7L2
ALOX12B	CCNE1	EGFR	FGF7	HIST1H3I	KMT2D	NEGR1	PMAIP1	RPTOR	TERC
ANKRD11	CD274	EIF1AX	FGF8	HIST1H3J	KRAS	NF1	PMS1	RUNX1	TERT
ANKRD26	CD276	EIF4A2	FGF9	HIST2H3A	LAMP1	NF2	PMS2	RUNX1T1	TET1
APC	CD74	EIF4E	FGFR1	HIST2H3C	LATS1	NFE2L2	PNRC1	RYBP	TET2
AR	CD79A	EML4	FGFR2	HIST2H3D	LATS2	NFKBIA	POLD1	SDHA	TFE3
ARAF	CD79B	EP300	FGFR3	HIST3H3	LMO1	NKX2-1	POLE	SDHAF2	TFRC
ARFRP1	CDC73	EPCAM	FGFR4	HLA-A	LRP1	NKX3-1	PPARG	SDHB	TGFBR1
ARID1A	CDH1	EPHA3	FH	HLA-B	LYN	NOTCH1	PPM1D	SDHC	TGFBR2
ARID1B	CDK12	EPHA5	FLCN	HLA-C	LZTR1	NOTCH2	PPP2R1A	SDHD	TMEM127
ARID2	CDK4	EPHA7	FLI1	HNF1A	MAGI2	NOTCH3	PPP2R2A	SETBP1	TMPRSS2
ARID5B	CDK6	EPHB1	FLT1	HNRNPK	MALT1	NOTCH4	PPP6C	SETD2	TNFAIP3
ASXL1	CDK8	ERBB2	FLT3	HOXB13	MAP2K1	NPM1	PRDM1	SF3B1	TNFRSF14
ASXL2	CDKN1A	ERBB3	FLT4	HRAS	MAP2K2	NRAS	PRES2	SH2B3	TOP1
ATM	CDKN1B	ERBB4	FOXA1	HSD3B1	MAP2K4	NRG1	PRKAR1A	SH2D1A	TOP2A
ATR	CDKN2A	ERCC1	FOXL2	HSP90AA1	MAP3K1	NSD1	PRKCI	SHQ1	TP53
ATRX	CDKN2B	ERCC2	FOXO1	ICOSLG	MAP3K13	NTRK1	PRKDC	SLIT2	TP63
AURKA	CDKN2C	ERCC3	FOXP1	ID3	MAP3K14	NTRK2	PRSS8	SLX4	TRAF2
AURKB	CEBPA	ERCC4	FRS2	IDH1	MAP3K4	NTRK3	PTCH1	SMAD2	TRAF7
AXIN1	CENPA	ERCC5	FUBP1	IDH2	MAPK1	NUP93	PTEN	SMAD3	TSC1
AXIN2	CHD2	ERG	FYN	IFNGR1	MAPK3	NUTM1	PTPN11	SMAD4	TSC2
AXL	CHD4	ERRFI1	GABRA6	IGF1	MAX	PAK1	PTPRD	SMARCA4	TSHR
B2M	CHEK1	ESR1	GATA1	IGF1R	MCL1	PAK3	PTPRS	SMARCB1	U2AF1
BAP1	CHEK2	ETS1	GATA2	IGF2	MDC1	PAK7	PTPRT	SMARCD1	VEGFA
BARD1	CIC	ETV1	GATA3	IKBKE	MDM2	PALB2	QKI	SMC1A	VHL
BBC3	CREBBP	ETV4	GATA4	IKZF1	MDM4	PARK2	RAB35	SMC3	VTCN1
BCL10	CRKL	ETV5	GATA6	IL10	MED12	PARP1	RAC1	SMO	WISP3
BCL2	CRLF2	ETV6	GEN1	IL7R	MEF2B	PAX3	RAD21	SNCAIP	WT1
BCL2L1	CSF1R	EWSR1	GID4	INHA	MEN1	PAX5	RAD50	SOCS1	XIAP
BCL2L11	CSF3R	EZH2	GLI1	INHBA	MET	PAX7	RAD51	SOX10	XPO1
BCL2L2	CSNK1A1	FAM123B	GNA11	INPP4A	MGA	PAX8	RAD51B	SOX17	XRCC2
BCL6	CTCF	FAM175A	GNA13	INPP4B	MITF	PBRM1	RAD51C	SOX2	YAP1
BCOR	CTLA4	FAM46C	GNAQ	INSR	MLH1	PDCD1	RAD51D	SOX9	YES1
BCORL1	CTNNA1	FANCA	GNAS	IRF2	MLL	PDCD1LG2	RAD52	SPEN	ZBTB2
BCR	CTNNB1	FANCC	GPR124	IRF4	MLLT3	PDGFRA	RAD54L	SPOP	ZBTB7A
BIRC3	CUL3	FANCD2	GPS2	IRS1	MPL	PDGFRB	RAF1	SPTA1	ZFX3
BLM	CUX1	FANCE	GREM1	IRS2	MRE11A	PDK1	RANBP2	SRC	ZNF217
BMPR1A	CXCR4	FANCF	GRIN2A	JAK1	MSH2	PDPK1	RARA	SRSF2	ZNF703
BRAF	CYLD	FANCG	GRM3	JAK2	MSH3	PGR	RASA1	STAG1	ZRSR2
BRCA1	DAXX	FANCI	GSK3B	JAK3	MSH6	PHF6	RB1	STAG2	
BRCA2	DCUN1D1	FANCL	H3F3A	JUN	MST1	PHOX2B	RBM10	STAT3	
BRD4	DDR2	FAS	H3F3B	KAT6A	MST1R	PIK3C2B	RECQL4	STAT4	
BRIP1	DDX41	FAT1	H3F3C	KDM5A	MTOR	PIK3C2G	REL	STAT5A	
BTG1	DHX15	FBXW7	HGF	KDM5C	MUTYH	PIK3C3	RET	STAT5B	
BTK	DICER1	FGF1	HIST1H1C	KDM6A	MYB	PIK3CA	RFWD2	STK11	
C11orf30	DIS3	FGF10	HIST1H2BD	KDR	MYC	PIK3CB	RHEB	STK40	

56 genes by RNA sequencing: • Fusions • Breakpoints • Splice variants

(All genes listed are assessed for known and novel fusions, In addition, the content shaded in grey is analyzed for splice variants.)

ABL1	BRAF	EML4	ETV4	FGFR4	KIF5B	MSH2	NRG1	PX7	RAF1
AKT3	BRCA1	ERBB2	ETV5	FLI1	KIT	MYC	NTRK1	PDGFRA	RET
ALK	BRCA2	ERG	EWSR1	FLT1	MET	NOTCH1	NTRK2	PDGFRB	ROS1
AR	CDK4	ESR1	FGFR1	FLT3	ML	NOTCH2	NTRK3	PIK3CA	RPS6KB1
AXL	CSF1R	ETS1	FGFR2	JAK2	MLLT3	NOTCH3	PAX3	PPARG	TMPRSS2
BCL2	EGFR	ETV1	FGFR3	KDR					

Sequencing Laboratory Methods:

DNA and RNA are isolated from formalin-fixed, paraffin-embedded (FFPE) tissues. Sequencing libraries are generated using the Illumina® TruSight™ Oncology 500 panel library preparation reagents. A panel of biotinylated single stranded probes are used to enrich the DNA derived libraries for all exons of 523 cancer related genes and 130 microsatellite instability loci. A separate panel of biotinylated single stranded probes are used to enrich the RNA derived libraries for all exons of 56 cancer-related genes. The enriched libraries are sequenced on Illumina® NextSeq™ and NovaSeq™ sequencing platforms.

Depth of coverage:

To generate the low coverage table, the laboratory calculates the percentage of the gene that is covered below 100X for the regions covered by the assay. A gene is listed as low coverage if 10% of its exon region is below 100x. The low coverage regions and a description of how they are calculated are included in the patient report.

Limitations:

Variants below the limit of detection, insertions/deletions >40 bp, and fusions that do not alter expressed messenger RNA may not be detected by this assay. A negative result does not exclude the presence of a variant beyond these detection limitations. The assay is not informative for mutations outside the 523 cancer-related genes or for those regions for which the assay achieves limited coverage. This assay is not validated for large (> 40bp) indels, complex structural variants, or gene-level deletion events. Further, the assay is not validated to detect fusions that do not alter the expressed transcript (such as those that occur in the MYC gene). Given the overlap between the RNA and DNA capture, a total of 523 genes are interrogated across the RNA and DNA components of the test. This test targets coding regions and may not detect intronic variants, including those that may affect splicing. MSI analysis can identify MSI Positive and MSI Negative; however, MSI-Low may not be detected. The threshold for reporting amplifications in genes is 2.5 fold normalized increase in coverage. Lower Limit of Detection (LLOD) that meet the lab's performance criteria is 5%. The lab can report below 5%, however, there is reduced sensitivity (PPA) below 5% and may not detect low VAF variants. Treatment recommendations are based on ASCO and NCCN guidelines, and other peer reviewed literature. All recommended therapies are FDA approved, both for the indication, and outside the indication where supported by guidelines or compendia.

REFERENCES:

1. Conroy et al. A scalable high-throughput targeted next-generation sequencing assay for comprehensive genomic profiling of solid tumors. *PLoS One*. 2021 Dec 2;16(12):e0260089.
2. Pestinger et al. Use of an Integrated Pan-Cancer Oncology Enrichment Next-Generation Sequencing Assay to Measure Tumour Mutational Burden and Detect Clinically Actionable Variants. *Mol Diagn Ther*. 2020 Jun;24(3):339-349.
3. Benayed et al. High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no mitogenic driver alteration as detected by DNA sequencing and low tumor mutation burden. *Clin Cancer Res*. 2019 August 01; 25(15):4712-4722.
4. Wong et al. Methods for Identifying Patients with Tropomyosin Receptor Kinase (TRK) Fusions. *Cancer*. *Pathol Oncol Res*. 2020 Jul; 26(3): 1385-1399.
5. Yang et al. A Performance Comparison of Commonly Used Assays to Detect RET Fusions. *Clin Cancer Res*. 2020 Dec 3.
6. Radonic et al. RET FISH Analysis is a sensitive by highly unspecific method for RET fusions in lung cancer. *J Thora Oncol*. 2021 Feb 12.
7. Wei B, Kang J, Kibukawa M, et al: Evaluation of the TruSight Oncology 500 Assay for Routine Clinical Testing of Tumor Mutational Burden and Clinical Utility for Predicting Response to Pembrolizumab. *The Journal of Molecular Diagnostics*.
8. Merino DM, McShane L, Butler M, et al: TMB standardization by alignment to reference standards: Phase II of the Friends of Cancer Research TMB Harmonization Project. *Journal of Clinical Oncology* 37:2624-2624, 2019 9. Merino DM, et al: Abstract 5671: Alignment of TMB measured on clinical samples: Phase IIB of the Friends of Cancer Research TMB Harmonization Project. *Cancer Research* 80, 2020.



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