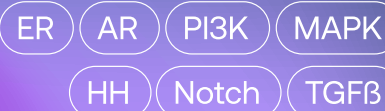


# oncosignal



The OncoSIGNal Pathway Activity Profiling G7 Test for measuring ER, AR, PI3K, MAPK, Hedgehog (HH), Notch and TGFβ pathway activity is available for research use in your molecular biology laboratory. The OncoSIGNal Test is performed using an RT-qPCR testing plate to measure mRNA levels of selected pathway target genes and includes access to a secure cloud-based environment for calculation and reporting of pathway activity scores.

OncoSIGNal is based on a unique knowledge-based approach and helps users gain insight into underlying tumor driving cell signaling pathways. The OncoSIGNal Test quantitatively measures the activity of the hormonal estrogen receptor (ER), androgen receptor (AR), MAPK, Hedgehog (HH) pathways, as well as the activity of Notch and TGFβ growth factor signal transduction pathways simultaneously in a single sample, using RNA from FFPE tissue of human origin.

## OncoSIGNal Testing Plates

mRNA levels transcribed from target genes regulated by the pathway transcription factor are measured by means of the OncoSIGNal Testing Plates. The 96-well PCR testing plates are packaged in a box of six, for testing of six samples. Each testing plate contains carefully designed and optimized primers and probes for reliable detection of mRNA levels of the pre-selected signaling pathway target genes. Upon adding the sample RNA and one-step RT-qPCR mix to the testing plate, the test is run on a thermocycler under a specified PCR cycling program. Detailed protocols are provided in the Instructions for Use.



## OncoSIGNal Report

PCR gene expression data are translated via a computational model into quantitative pathway activity scores. Each PCR data file uploaded in the cloud-based ISPM OncoSIGNal portal undergoes a thorough quality check (QC) (e.g. controls for the quantity and quality of the RNA, and detection of correct plate filling), to ensure high-quality results. The OncoSIGNal Report presents an overview of the pathway activity scores per sample on a scale of 0-100 with respective 95% confidence intervals. The report provides insights into the tumor driving pathways in the samples tested and enables a direct comparison of pathway activities between samples.

Sample Information				Flag	Pathway activity score (CI=95% confidence interval)											
Sample ID	Tissue type	Tumor cell%	QC	ER	CI lower	CI upper	AR	CI lower	CI upper	PI3K <sup>1</sup>	CI lower	CI upper	MAPK <sup>2</sup>	CI lower	CI upper	
102369	pre-treatment	Breast	60	Passed	57	55	61	20	18	22	75	70	80	11	10	14
102370	post-treatment (AI)	Breast	65	Passed	23	21	25	26	24	28	82	79	85	11	9	14
102371	pre-treatment	Breast	50	Passed	85	83	88	36	30	42	38	35	41	43	40	45
102372	post-treatment (AI)	Breast	80	Passed	16	13	18	35	33	38	32	30	33	47	40	44
102373	pre-treatment	Breast	80	Passed	28	26	30	17	15	18	17	15	18	55	53	30
102374	post-treatment (AI)	Breast	80	Passed	26	24	28	11	10	13	19	18	21	50	44	56

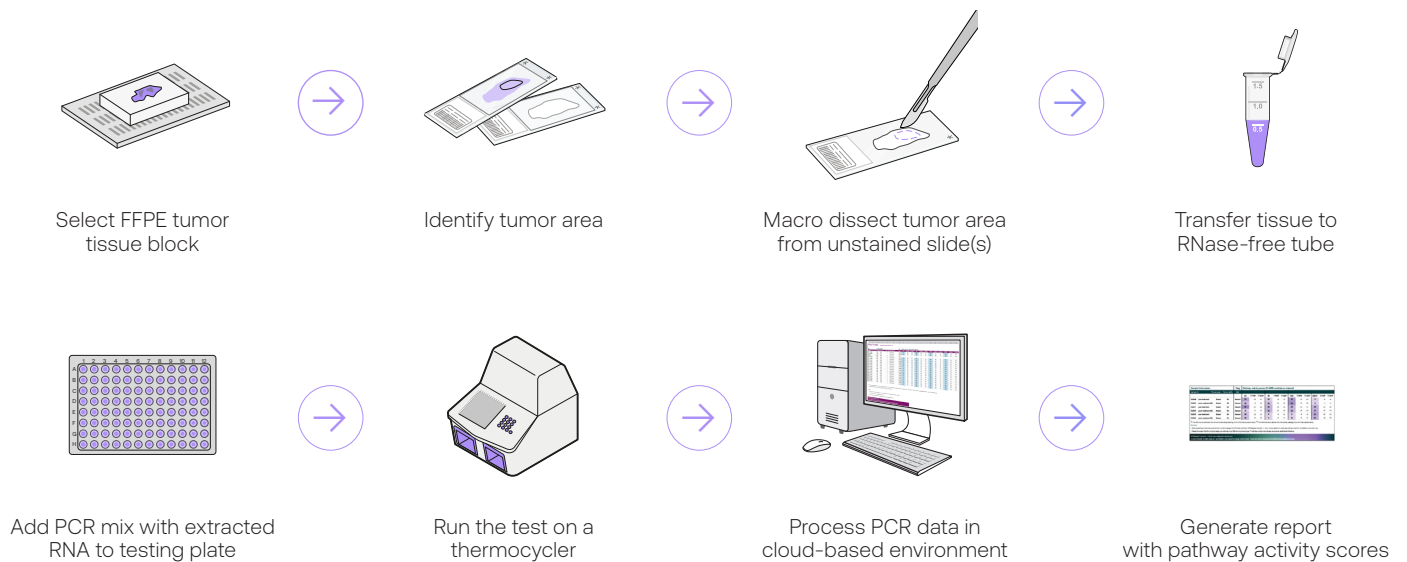
<sup>(1)</sup> The PI3K score is derived from the inverse activity reading of the FOXO transcription factor. <sup>(2)</sup> The MAPK score is derived from the activity reading of the AP-1 transcription factor.

Remarks:  
 - Pathway activity scores are presented on a scale ranging from 0 (lowest activity) to 100 (highest activity). - A color scale is applied on pathway activity scores for visualization purposes only.  
 - Please be aware that the biological range per pathway may differ among tissue types. Therefore, a single result always requires an established reference. - Report is an example of four pathways activity scores.

For Research Use only – not for use in diagnostic procedures.

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## Workflow OncoSIGNal Test



### Features

- AR, ER, PI3K, MAPK, Hedgehog, Notch, TGF $\beta$  pathway activity
- Highly reproducible quantitative pathway activity scores on a scale of 0-100 with confidence intervals
- Translation of RT-qPCR data into pathway activity scores in a secure cloud-based environment
- Report providing pathway activity profile per sample
- Easy to implement in molecular biology laboratory
- Requires only 0.35 mm<sup>3</sup> FFPE tissue per sample
- Fast turn-around-time (105 minutes)

### Box with OncoSIGNal Testing Plates contains

- Six OncoSIGNal Testing Plates (96-wells plates with pre-designed and spotted qPCR assays)
- Six (+one spare) clear, adhesive PCR plate foils
- Quick Reference Guide

### Order information

Please send your request to [oncosignal@innosignbio.com](mailto:oncosignal@innosignbio.com).

More information about the OncoSIGNal Test can be found at [www.oncosignal.com](http://www.oncosignal.com).

### Additional materials needed

- RNeasy FFPE Kit (Qiagen, cat.no. 73504)
- Deparaffinization Solution (recommended) (Qiagen, cat.no. 19093)
- SuperScript III Platinum One-Step qRT-PCR Kit (Thermo Fisher Scientific, cat.no. 11732088)
- Nuclease-free water
- Bio-Rad CFX96 (Touch) Real-Time PCR Detection System + Maestro Software
- Plate centrifuge
- Repeater pipet (recommended)
- Dedicated roller or alternative to seal foil (see IfU)

### Applications

For information about example experimental designs, and interpretation of the OncoSIGNal Report, please request the application note.