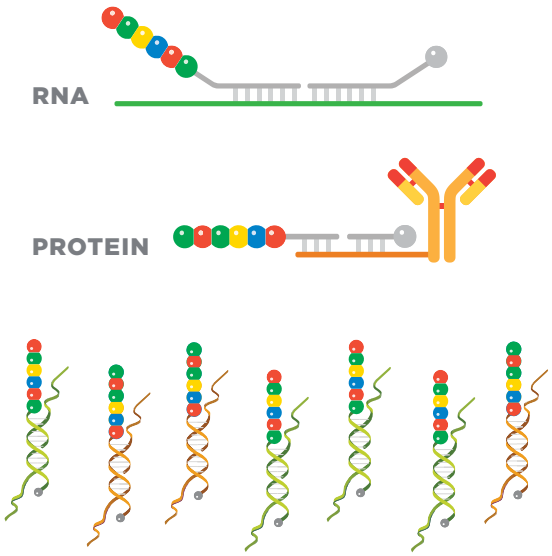
nCounter® Vantage 3D™ RNA:Protein Solid Tumor Assay

Simplify Tumor Characterization with Multiplexed Detection of RNA, Protein, and Phospho-Protein Expression in Key Signaling Pathways

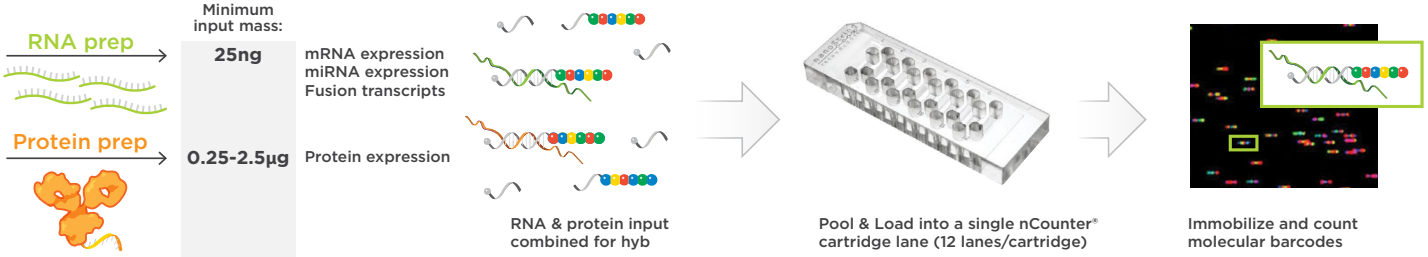
The nCounter® Vantage 3D™ RNA:Protein Solid Tumor Assay simplifies RNA and protein expression analysis with curated content covering 770 RNA from the PanCancer Pathways panel and up to 28 total and phospho-proteins. This highly multiplexed assay is capable of simultaneously characterizing RNA and protein expression from as little as 1 µg of protein or just 2 FFPE slides (protein-only analysis requires 250 ng or 1 FFPE slide). Built on the core nCounter® technology, which uses unique molecular barcodes to detect nucleic acids of increasing variety, NanoString protein analysis utilizes primary antibodies specific to proteins of interest that have been barcoded with unique synthetic DNA oligonucleotides. Each DNA oligonucleotide is recognized by a unique reporter probe that contains a fluorescent barcode. Reporter probes are imaged and counted by the nCounter Analysis System to provide a direct, digital readout of protein expression. Designed with 3D Biology™ Technology, the Vantage 3D Protein Solid Tumor Panel delivers reliable protein expression profiling that may be combined with other Vantage 3D RNA or Fusion Gene Assays, PanCancer RNA Panels, and Custom CodeSets to provide customized and flexible tumor characterization.



Product Highlights

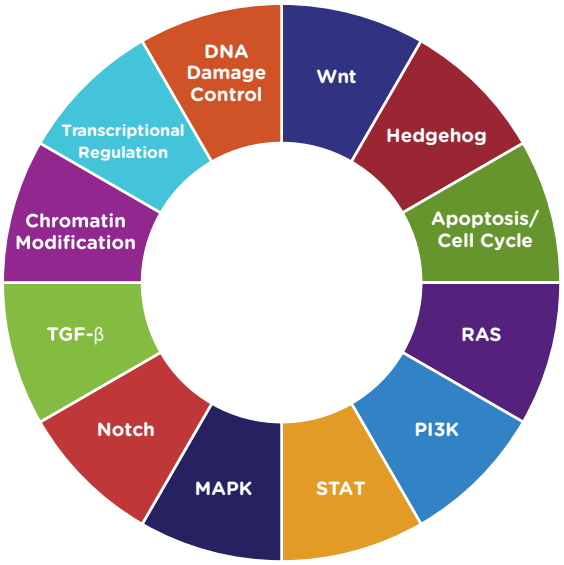
- More data from your precious samples; RNA:Protein profiling requires just 1 µg of protein or 2 FFPE slides (protein-only analysis requires 250 ng or 1 FFPE slide)
- Simple protocol for FFPE (RNA and protein) and both cell and tissue lysates (RNA)
- High concordance with gold standard protein analysis techniques
- Simple, integrated data analysis, eliminating the need for a bioinformatician
- Customizable with up to 5 additional protein targets of interest
- Flexible design enables mix and match capabilities with other nCounter Vantage 3D Assays

1. Prepare Sample | **2. Hybridize** | **3. Pool & Load** | **4. Analyze/report**



Curated Content Focused on Key Signaling Pathways

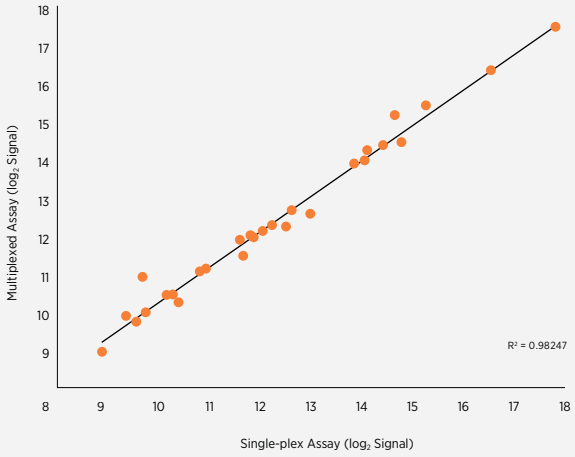
The Vantage 3D Protein Solid Tumor content was selected to pair with and enhance the PanCancer Pathways RNA panel, which was developed using a biology-guided, data-driven approach, and basic co-expression principles were applied to score and rank each gene based on its biological relevance to cancer and its role as an essential representation in one or more of the 13 canonical pathways (highlighted to the right). The curated total and phospho-protein content focuses on a subset of these canonical pathways, highlighted below.



Protein Targets	Driver Gene	MAPK	PI3K	Ras	Cell Cycle
4E-BP1/Phospho-4E-BP1 (Thr37/46)	-	-	+	-	-
EGF Receptor/Phospho-EGF Receptor (Tyr1068)	+	+	+	+	-
GSK-3β/Phospho-GSK-3β (Ser9)	-	-	+	-	+
HER2/ErbB2	+	+	+	+	-
Ki-67	-	-	-	-	+
Met	+	-	+	+	-
ERK/Phospho-ERK (Thr202/Tyr204)	-	+	+	+	-
p53	+	+	+	-	+
Akt/Phospho-Akt (Ser473)	+	+	+	+	+
Keratin	-	+	-	-	-
Phospho-AMPKα (Thr172)	-	-	+	-	-
Phospho-Chk1 (Ser345)	-	-	-	-	-
Phospho-c-Raf (Ser259)	-	+	+	+	-
Phospho-Histone H3 (Ser10)	-	-	-	-	+
Phospho-MEK1/2 (Ser217/221)	+	+	+	+	-
Phospho-p70 S6 Kinase (Thr389)	-	-	+	-	-
Phospho-PDK1 (Ser241)	-	-	+	-	-
Phospho-PRAS40 (Thr246)	-	-	+	-	-
Progesterone Receptor	+	-	-	-	-
S6/Phospho-S6 Ribosomal Protein (Ser235/236)	-	-	+	-	-
Tuberin/TSC2/Phospho-Tuberin/TSC2 (Thr1462)	-	-	+	-	-

Protein Detection with High Specificity

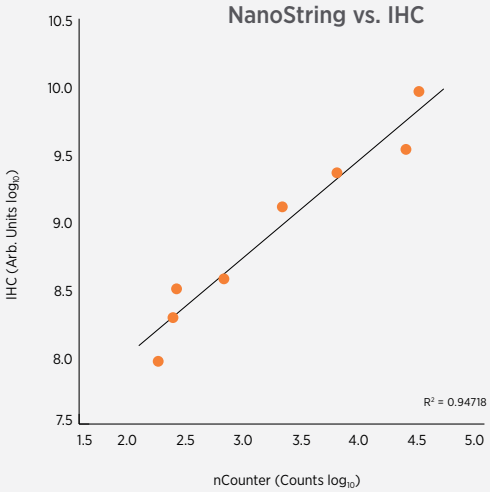
Protein detection shows high specificity with no change in signal for single-plex versus multiplex assay formats.



All protein targets are tested in single-plex and multiplex format to ensure consistent detection in the multiplex format.

Concordance with Gold Standard Techniques

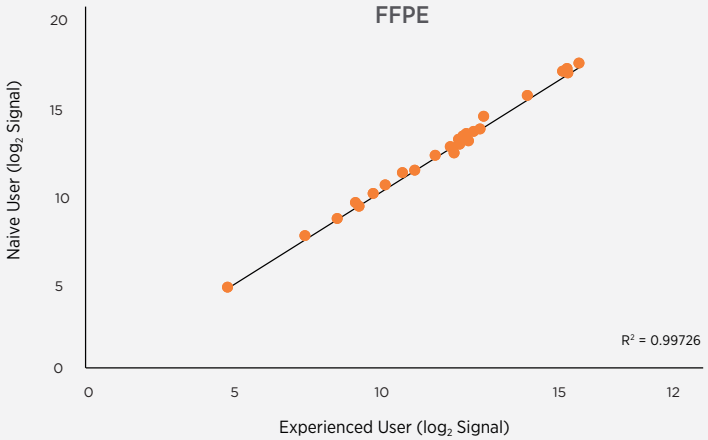
The nCounter Vantage 3D Protein Solid Tumor Panels show high concordance with gold standard techniques.



Quantification of Her2 expression by IHC versus NanoString across 8 samples with varying expression profiles.

Reproducibility across Sample Types

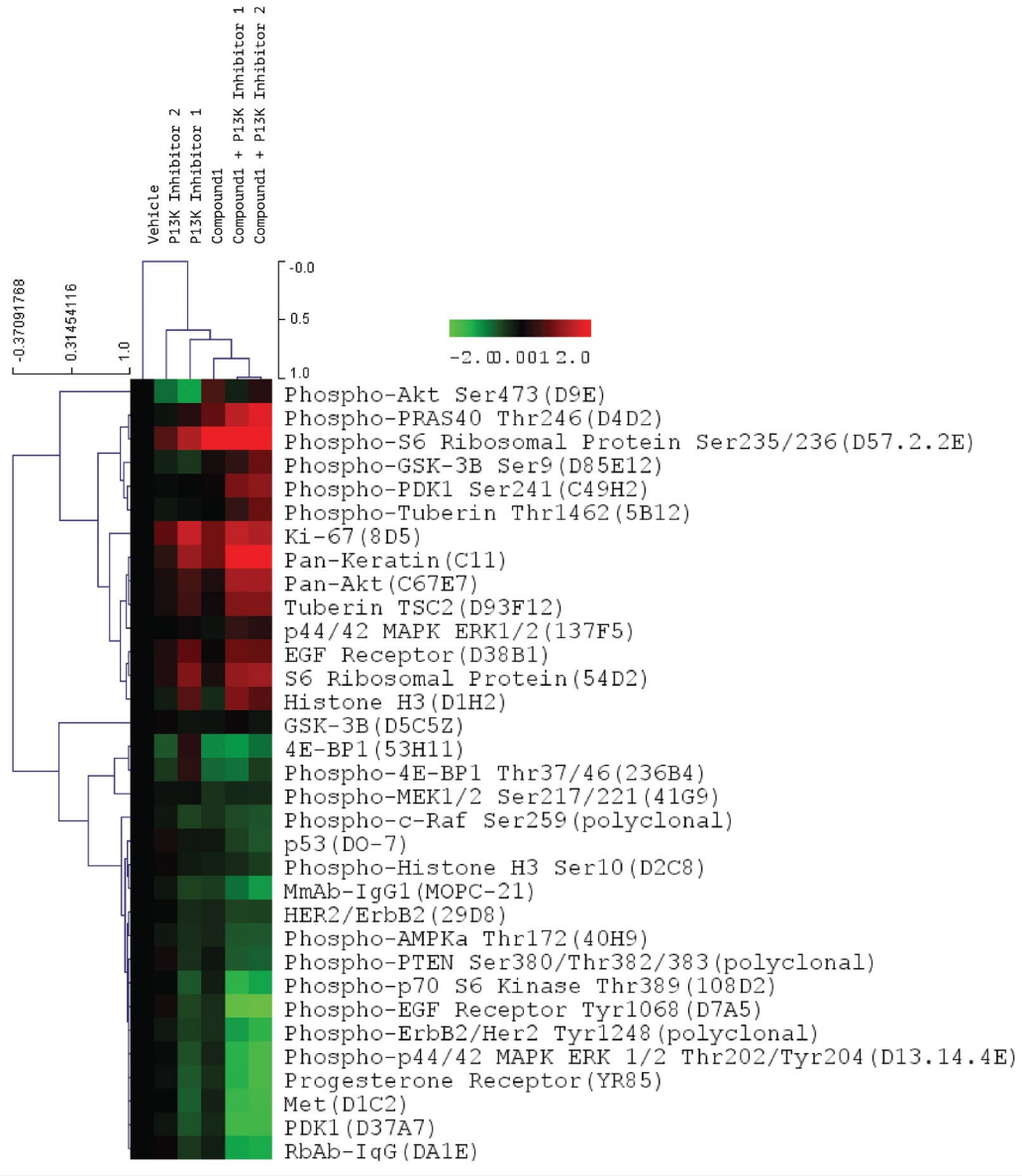
Both RNA and protein expression profiles show high reproducibility between users for both lysate and FFPE profiling, providing confidence in your data.



Samples run by first-time users versus an experienced assay user show Vantage 3D Solid Tumor Protein expression profiles with high correlation, demonstrating assay robustness.

Multiplex Profiling across Key Signaling Cascades

Multiplex RNA:Protein detection allows you to track expression response across signaling pathways simultaneously. For example, the RNA:Protein response was profiled after treatment with Compound 1 +/- PI3K inhibition. Protein expression response clusters by inhibitor treatment and PI3K inhibition shows a reduction in phoshpho-Akt, but in combination with Compound 1 increases phospho-PRAS40, for example.



Multiplex protein expression profile in cell lines highlights key signaling cascades that are upregulated or downregulated by specific treatments.

Ordering Information

Name	Format	Description	Quantity	Catalog Number
nCounter Vantage 3D RNA:Protein Solid Tumor Assay for FFPE	Code Set Only	Prematched RNA:Protein assay	12 reactions	VDRPC-SPKP-HSTF-12
nCounter Vantage 3D Protein Solid Tumor Panel for FFPE (D)	Code Set Only	For use as a stand-alone protein assay or with nCounter Vantage 3D DNA	12 reactions	VPODC-SPKP-HSTF-12
nCounter Vantage 3D Protein Solid Tumor Panel for FFPE (R)	Code Set Only	For use with RNA (+/- DNA) including custom CodeSets, PanCancer RNA Panels, and nCounter Vantage 3D RNA	12 reactions	VPRXC-SPKP-HSTF-12
nCounter Vantage 3D Protein Barcoding Service	Service	Customization Service for spike-in to above protein panels or full custom panel development	1 each	PBS-1AB
nCounter Vantage 3D Protein Barcoding Service TagSet (D)	Tag Set Only	Protein detection reagent to support a custom stand-alone protein assay or custom protein for use with nCounter Vantage 3D DNA	12 reactions	PBS-POD-12
nCounter Vantage 3D Protein Barcoding Service TagSet (R)	Tag Set Only	Protein detection reagent to support custom protein use with RNA (+/-DNA) including custom CodeSets, PanCancer RNA Panels, and nCounter Vantage 3D RNA Panels	12 reactions	PBS-PXR-12
nCounter Vantage 3D Protein Barcoding Service - Cross Reactivity Testing	Service	Cross Reactivity Service for spike-in custom protein only	1 each	PBS-XTEST
nCounter Master Kit (Max or Flex Systems)	Reagents and Cartridges	Reagents, cartridges, and consumables necessary for sample processing	12 reactions	NAA-AKIT-012
nCounter SPRINT™ Cartridge	Reagents and Cartridges	1 Cartridge, 12 lanes	12 reactions	SPRINT-CAR-1.0
nCounter SPRINT™ Reagent Pack	Reagents and Cartridges	Reagents A,B,C, & Hybridization Buffer	192 reactions	SPRINT-REAG-KIT

3D BIOLOGY™

THE END OF PROFILING AS YOU KNOW IT.

Stop asking, “What can I measure?” Start asking, “What should I measure?” Thanks to nCounter Vantage 3D assays, you get the full picture—targeted DNA, RNA, and Protein analysis in one process. Learn more at: nanostring.com/3D

For more information, please visit nanostring.com

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