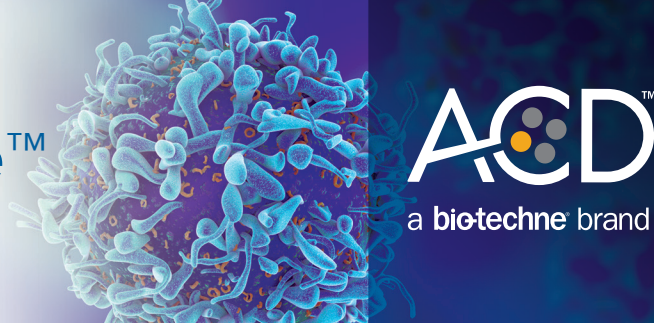
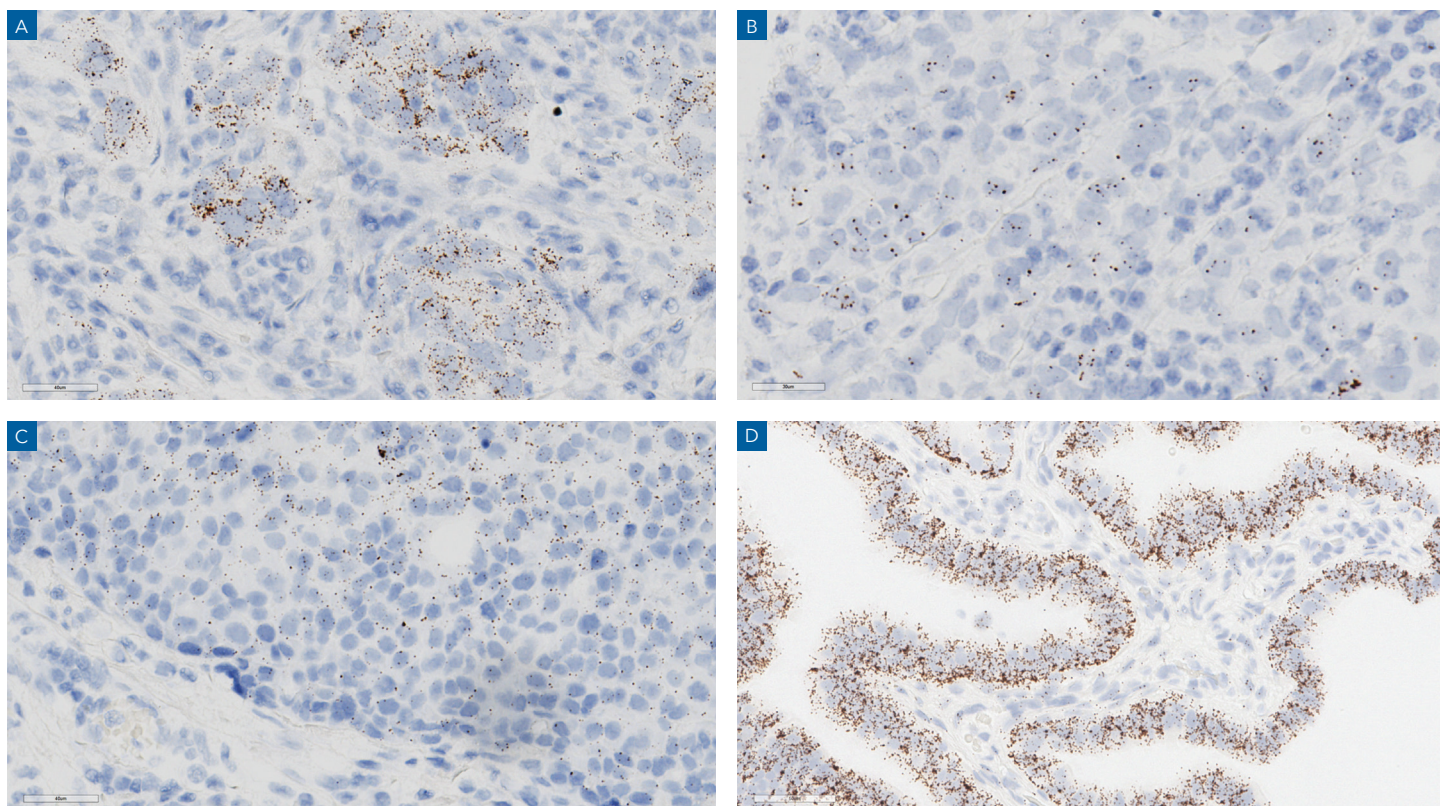


# WT1 Detection by RNAscope™ in Human Cancers



Wilms' tumor gene (*WT1*) is expressed in various human malignancies, including both hematological and solid cancers. While expression is found in normal tissues, overexpression of *WT1* is a common feature in various cancers as its protein product is a potent transcriptional regulator acting as a tumor suppressor. Antibody-based detection of transcription factors has been historically difficult due to the rigid structure of protein-protein interactions, which may mask unidentified structural configurations. The RNAscope RNA *In Situ* Hybridization Platform, including chromogenic and fluorescent detection reagents and target-specific probes, allows for the detection of *WT1* at the single mRNA molecule level in individual cells, all while providing spatial and morphological context. The double Z probe design allows for simultaneous signal amplification and suppression of non-specific background staining, ensuring target-specific detection.

**Figure 1:** RNAscope detection of *WT1* in normal and malignant tissues



**Image Footnote:**

- A - Papillary serous adenocarcinoma of uterus (uterine cancer) - high expression
- B - Papillary serous adenocarcinoma of uterus (uterine cancer) - low expression
- C - Infiltrating ductal carcinoma (breast cancer)
- D - Normal fallopian tube

Expression of *WT1* was interrogated in both tumor and normal archival Tissue Microarrays (TMA) as well as cell lines on a Cell Pellet Array (CPA) using RNAscope 2.5 Brown Detection on an automated instrument using standard pretreatment conditions. The types of tumor and normal tissues included on the TMAs are listed in Table 1 and Table 2, respectively. The cell lines included in the CPA are listed in Table 3.



# Materials and Methods

## Study Samples

- Tumor TMA (120 cores, 1 slide)
- Normal TMAs (75 cores , 2 slides)
- Cell Pellet Array (60 cores, 2 slides)

## Instrument, Assay, and Protocol

Instrument: Leica BOND RX

RNAscope Assay

- BOND RNAscope Detection Reagents - Brown (Cat. No. 201000)

Target Probe: HS-WT1 (Cat. No. 415588)

Standard RNAscope LS Brown assay pretreatment conditions were used as follows:

- Staining Protocol: RNAscope DAB ISH
- Preparation: Bake and Dewax
- Protease: 15 mins @ 40°C
- HIER: RNAscope Target Retrieval 15 mins @ 95°C

## Expression of WT1 in CPA

Figure 2: RNAscope detection of WT1 in various cell lines

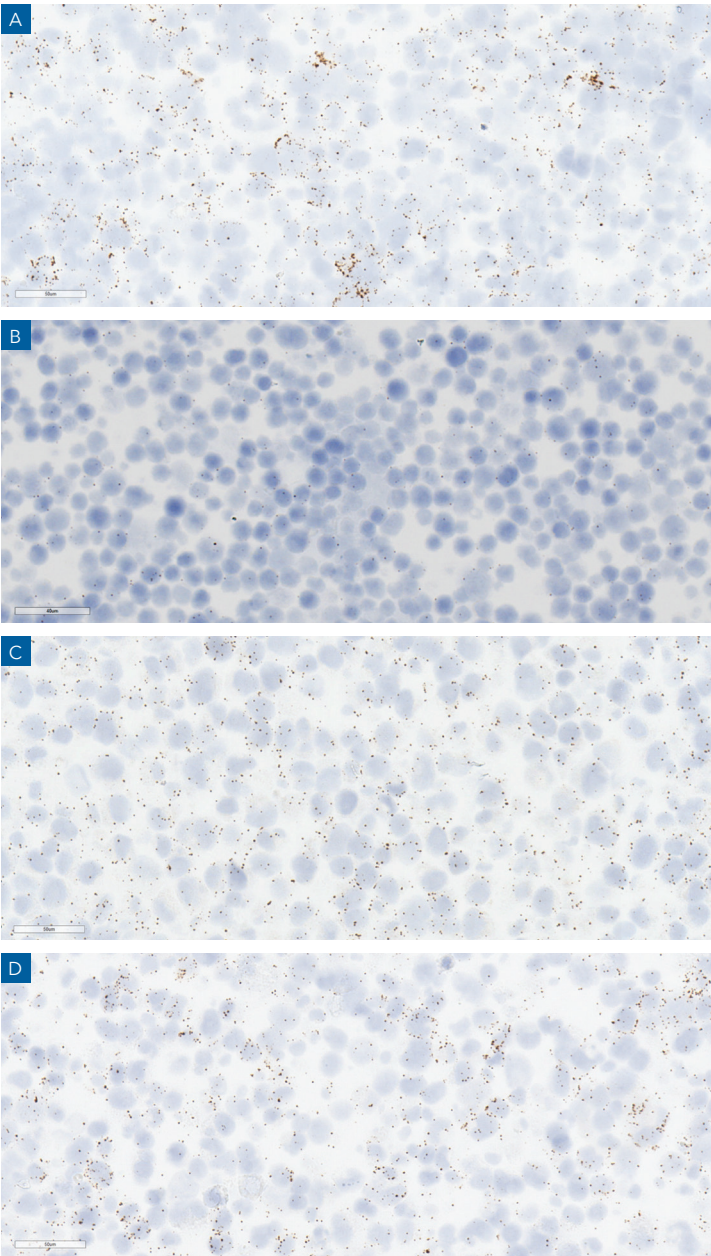


Table 1: Cell lines included on CPA

PANEL 1		PANEL 2	
Tissue Type	Cell Line Name	Tissue Type	Cell Line Name
Breast Cancer	HS-578T	Breast Cancer	SF-295
	BT-549		PFSK-1
	MCF7		A172
	T-47D		SW1088
	MDA-MB-231		DAOY
	MDA-MB-468		U251-MG
Lung Cancer	H23	Skin Cancer	UACC-62
	H226		A431
	H322		MALME-3M
	H460		M14
	H522		SK-MEL-2
	A549		SK-MEL-5
	EKVX		SK-MEL-28
	HOP-62	Gastric Cancer	SNU-16
Prostate Cancer	HOP-92		SNU-1
	PC-3	Pancreatic Cancer	KATOIII
	DU-145		MIAPACA2
	LNCAP		PANC1
Colon Cancer	22RV1	Leukemia	BXPC3
	SNU-C1		RPMI-8226
	COLO205		HL-60
	HT29		MOLT-4
	SW-620	Renal Cancer	CCRF-CEM
Ovarian Cancer	HCT-15		K-562
	HCT-116		A498
	SK-OV-3		CAKI-1
	IGR-OV1		ACHN
	PA-1		786-O
	CAOV-3		
	SW626		
	OVCAR-3		
	OVCAR-8		

Green Shaded Lines: RT-PCR analysis was performed

### Image Footnote:

- A - PFSK-1 (glial tumor)
- B - COLO 205 (colon cancer)
- C - UACC-62 (skin cancer)
- D - H23 (lung cancer)



# Expression of WT1 in Tumor TMA

Figure 3: RNAscope detection of WT1 in human cancers

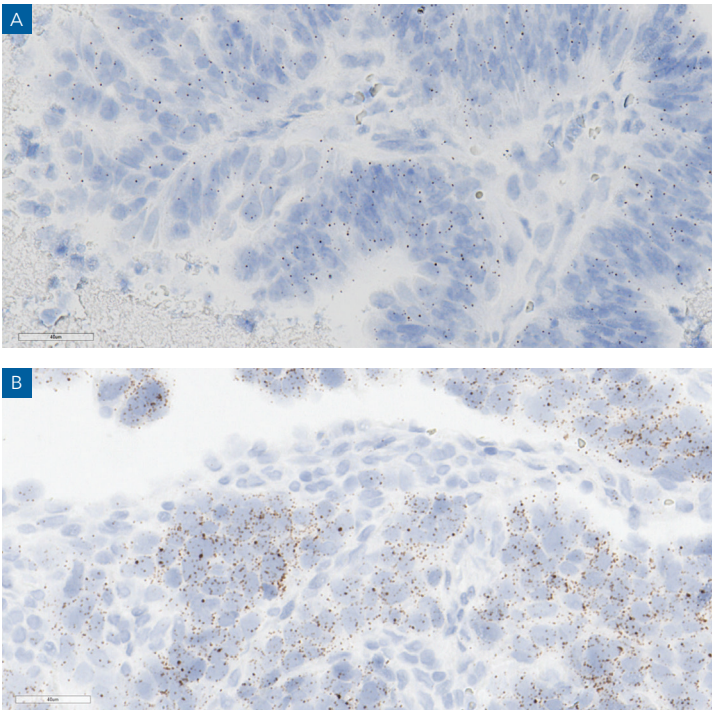


Image Footnote:  
A - Colorectal adenocarcinoma  
B - Papillary serous adenocarcinoma of uterus (uterine cancer)

# Expression of WT1 in Normal TMA

Figure 4: RNAscope detection of WT1 in normal human tissues

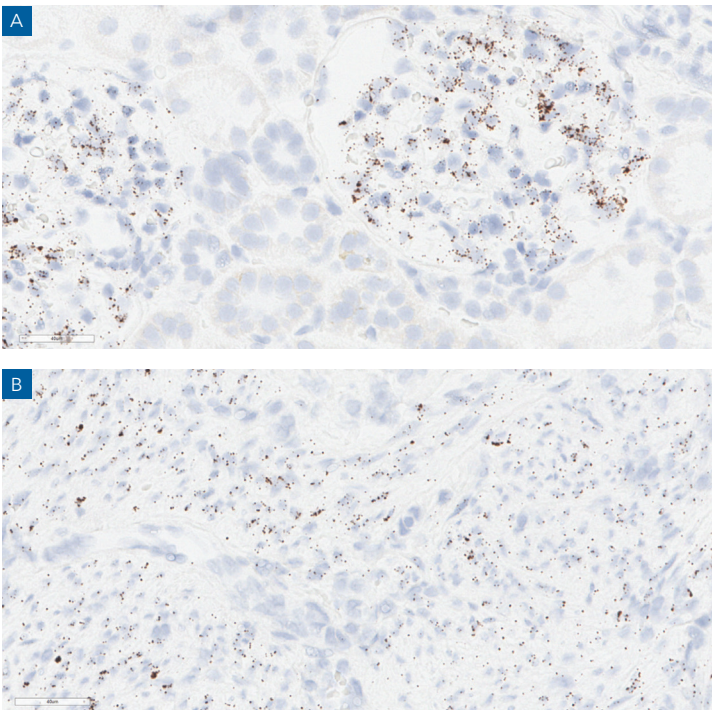


Image Footnote:  
A - Kidney (glomeruli)  
B - Uterus (myometrium)

Table 2: Cancer types included in our tumor TMA

Breast	Bladder	Colorectal	Head and Neck	Ovary	Lung
Prostate	Melanoma	Kidney	Pancreas		

Figure 6: Tumor TMA layout

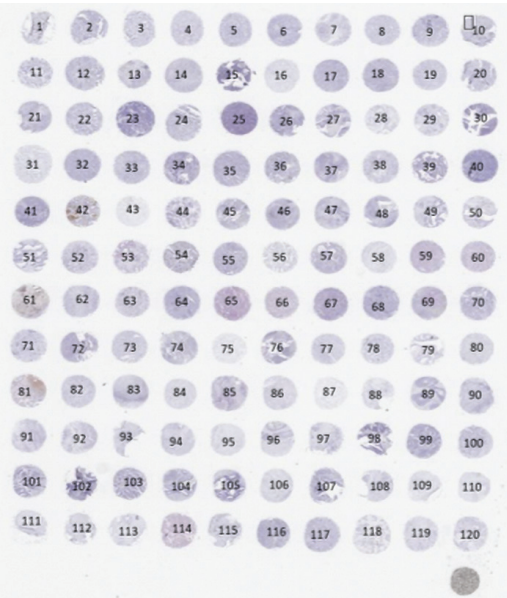
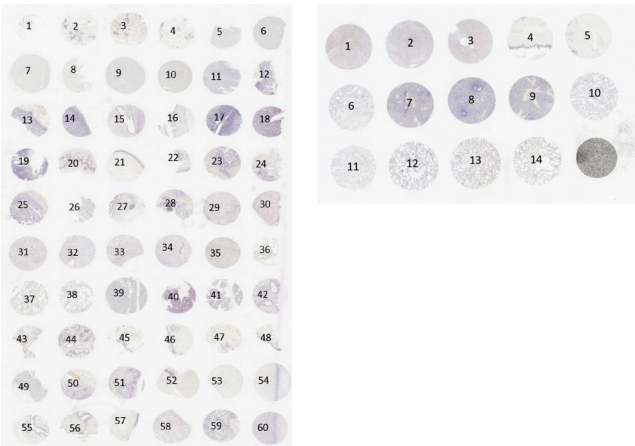


Table 3: Tissues included in normal TMAs

Adrenal Gland	Breast	Bladder	Cerebellum
Cerebrum	Colon	Cervix	Fallopian Tube
Gallbladder	Kidney	Liver	Lung
Pancreas	Prostate		
Seminal Vesicle	Skin	Spleen	Stomach
Testis	Thyroid	Uterus	

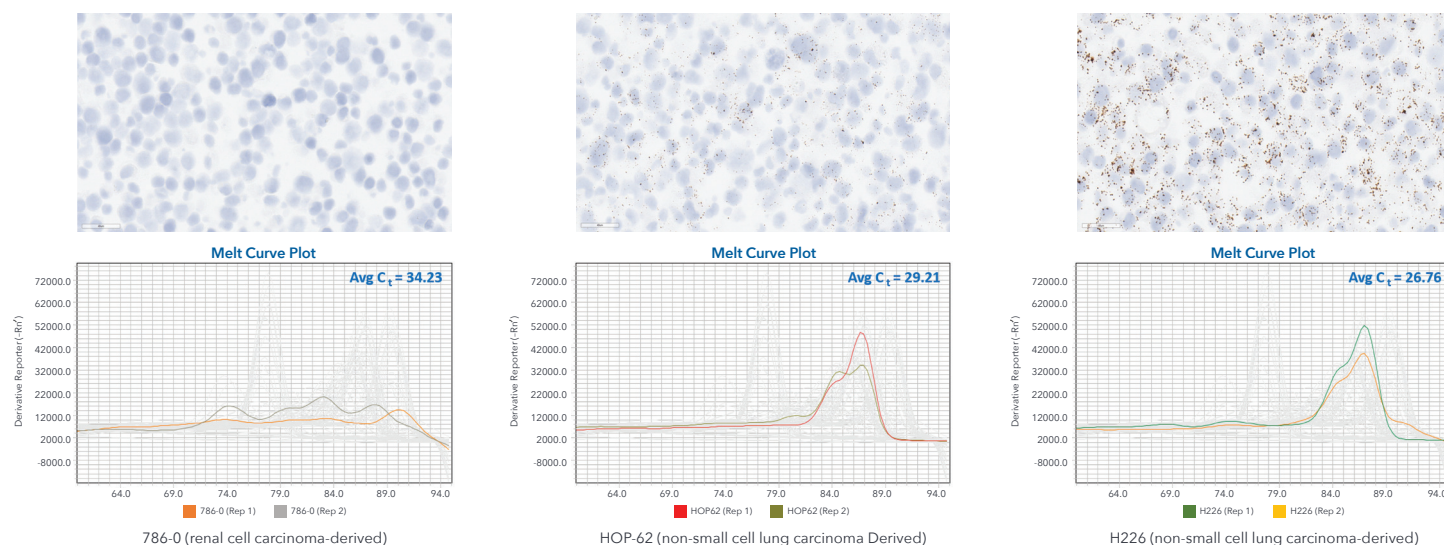
Figure 9: Normal TMA schematic



## Cell Pellet Expression Analysis: RNAscope Validation with qPCR

Orthogonal validation of RNAscope vs. RT-PCR for *WT1* expression was performed, demonstrating the specificity and quantitative nature of the RNAscope methodology (Figure 11). Expression by RNAscope RNA ISH was compared with qPCR across multiple cell pellet samples. RNAscope dot number was found to correlate with RT-PCR quantitation for *WT1*.

**Figure 11:** RNAscope *In Situ* Hybridization detects *WT1* mRNA expression levels that correlate with RT-PCR for *WT1* while providing tissue context and morphology



## Identify Therapeutic Biomarkers with RNAscope with Pharma Assay Services

The RNAscope *In Situ* Hybridization Platform, including detection reagents and target-specific probes, is a robust technology that allows for the identification of RNA expression patterns and localization at the single cell level with spatial and morphologic context. RNAscope is highly sensitive and specific due to its double Z probe design, resulting in an extremely high signal-to-noise ratio of staining in FFPE tissues relative to traditional RNA ISH, allowing researchers to visualize, localize, and quantify biomarker expression simultaneously. The technology is readily available on automated staining platforms, including the Leica Bond and Ventana Ultra platforms, for ease of use, high reproducibility, and seamless fit into the research laboratory workflow. Furthermore, RNAscope provides labs with the opportunity to add new biomarkers to their experiments and provide better options for problematic IHC antibodies. See for yourself with the *WT1* Dataset, which includes *WT1* expression in various tumors (120 TMA cores), normal tissues (75 TMA cores), and cell lines (60 CPA cores).

### Partner with ACD's Pharma Assay Services

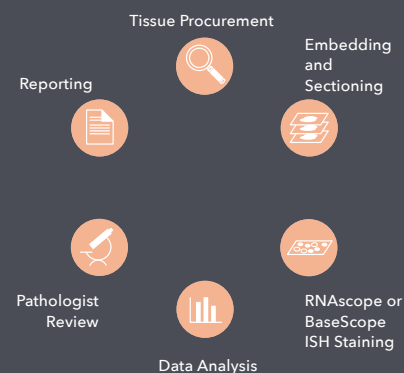
- Trust your study to the experts in RNAscope and BaseScope ISH
- Have confidence in the science, data and research conclusions
- Receive actionable results in weeks rather than months

"We've had a great experience in working with ACD. Using their service team we are able to move faster through our testing for Phase I trial, we are very happy with the quality of data, thoroughness in the reports we receive and would highly recommend them for ISH assay development and implementation."

– Dr. Omar Kabbarah

Learn more about Pharma Assay Services at [www.acdbio.com/PAS](http://www.acdbio.com/PAS)

pharma  
TOP 10  
CROs - 2020



To request a quote, contact: [acd\\_sales@bio-techne.com](mailto:acd_sales@bio-techne.com)

7707 Gateway Boulevard, Newark, CA 94560 | 1.510.576.8800 (Main) | 1.877.576.3636 (Toll Free)

For Research Use Only. Not for diagnostic use. RNAscope and BaseScope are trademarks of Advanced Cell Diagnostics, Inc. in the United States or other countries. All rights reserved. 2020 Advanced Cell Diagnostics, Inc. Doc #: MK 51-144/Rev A/Effective Date 3/30/2020