

**Spatial Biology**

# **Spatial RNA and Protein Detection**

Automated on Roche  
Discovery Ultra Platform



**biotechne®**

# Discover Automated RNA *in situ* Hybridization (ISH) Assays

Advanced Cell Diagnostics (ACD), a Bio-Techne brand, has partnered with Roche Tissue Diagnostics to offer the most robust *in situ* hybridization (ISH) assays on the market today - RNAscope™ and BaseScope™, automated on the Roche DISCOVERY ULTRA platform.

The combination of ACD's industry-leading *in situ* hybridization (ISH) technology and Roche's automated platform enable scientists to accelerate their research by minimizing hands-on time and to answer a wide range of research questions with comprehensive assay portfolio.


The assay delivers spatial and morphological context of targeted RNA expression at the single cell and sub-cellular resolution.

RNAscope and BaseScope VS automated assays are ideal for applications that can be difficult to achieve with other technologies. The assays can detect virtually any gene, in any organism and in any tissue.

- RNAscope VS assays are the reference standard in spatial molecular pathology for the widest range of samples. The assays are ideal for co-localization studies mapping the RNA expression of two targets in the same cell.
- BaseScope VS assay is based on proven RNA technology and is ideal for the study of exon junctions and splice variants, detecting the presence of small insertions or deletions within transcripts, and determining relationships among complex, highly homologous gene families.

RNA-Protein Co-Detection workflow enables researchers to simultaneously visualize RNA and Protein expression. The assays utilize ACD's patented RNAscope and BaseScope signal amplification and background suppression technologies to deliver supreme specificity and sensitivity with optimal signal-to-noise ratio. The integrated co-detection workflow (ICW) allows inclusion of virtually any antibodies to be combined with RNA ISH technology, enabling researchers to acquire more data and conserve precious samples.





***“The level of sensitivity of the assays allows for high quality and specificity. Moreover, the technical assistance for troubleshooting and support is phenomenal. I would highly recommend using ACD’s RNAscope technology with the DISCOVERY series of instruments.”***

**- Vivian Barry, Research Scientist**

# RNAScope ISH

## Automated Assays

### RNASCOPE AUTOMATED ASSAYS ON ROCHE DISCOVERY ULTRA PLATFORM

The RNAScope VS Universal HRP assay is a robust assay that delivers reliable results. It is the reference standard in spatial molecular pathology for the widest range of samples. In addition, the results can be visible under any standard bright field microscope or shared using digital pathology. The assay reagents are available in both Chromogenic (DAB, TEAL, PURPLE, GREEN) and Fluorescent (FITC, FAM, RED 610, Rhodamine, Rhodamine 6G, DCC, CY5) options and can be utilized when working with dark background or low express genes.

The RNAScope VS Universal AP assay when used with the Fast Red dye, offers excellent contrast for staining highly pigmented lung, liver, retina and melanoma tissue specimens. For genes where a lower expression is assumed, this assay is recommended because the red dots clearly distinguish against the hematoxylin staining and are visible under standard brightfield microscope.

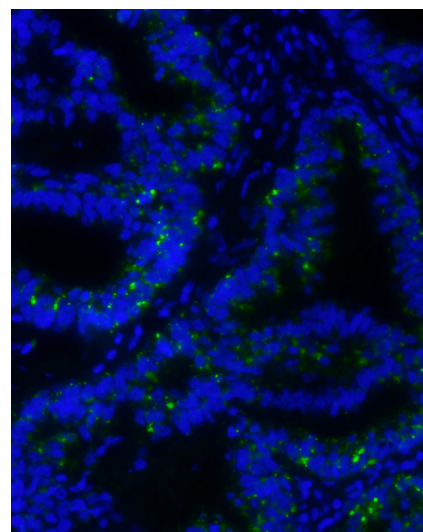


FIGURE 1. Detection of *PPIB* (FITC) in human colon cancer Fluorescent ISH (Singleplex) using RNAScope VS HRP and DISCOVERY FITC kit.

### RNASCOPE VS DUPLEX ASSAY

The RNAScope VS Duplex assay is designed for simultaneous detection of two RNA species. The assay is ideal for co-localization studies mapping the RNA expression of two targets in the same cell such as a secreted ligand and its cell-surface receptor.

The results are visualized under bright-field microscope. The probes and staining methods are used to distinguish between two probes.

- Channel 1 detects an HRP-based reaction producing a dark-brown, teal, or green signal (depending on the dye chosen).
- Channel 2 detects an AP-based Fast Red reaction resulting in a red color.

Due to the brightness of the red chromogen, the lower-expressing target is typically measured via the C2 channel with the stronger expressing target in the C1 channel.

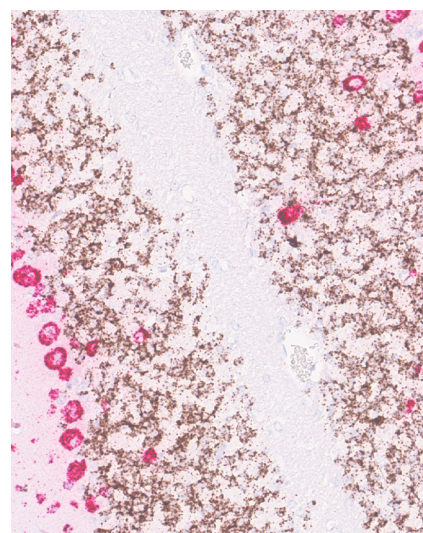


FIGURE 2. Detection *Nmdar1* (Brown) and *Ampa1* (Red) in mouse brain using RNAScope VS Duplex Reagent Kit and the DISCOVERY ULTRA platform from Roche Tissue Diagnostics.

# BaseScope ISH Automated Assay

## AUTOMATED SPLICE VARIANT & SHORT TARGET RNA DETECTION

BaseScope assay provides single-molecule detection of RNA targets as short as 50 bases in any tissue and across a variety of sample types.

Based on the same proven RNA technology, BaseScope's expanded capabilities are ideal for the study of exon junctions and splice variants, detecting the presence of small insertions or deletions within transcripts, and determining relationships among complex, highly homologous gene families.

BaseScope's 50-300 base length range is ideal for the following applications:

- Research involving circular RNA or gene fusions.
- Validation of knockouts and other CRISPR gene editing
- Detection of pre-miRNA, and the analysis of T cell receptors (TCRs) and CDR sequences in T-cell clones.

## KEY FEATURES

- Reliable results with sensitive and specific detection of short targets, highly homologous sequences, and splice variants
- Fully automated assay workflow on DISCOVERY ULTRA system saves hands-on time and minimizes error
- Broad sample-type compatibility that includes FFPE, fixed-frozen, and fresh frozen tissues
- Easy visualization with high-contrast Fast Red dye using standard brightfield microscopy

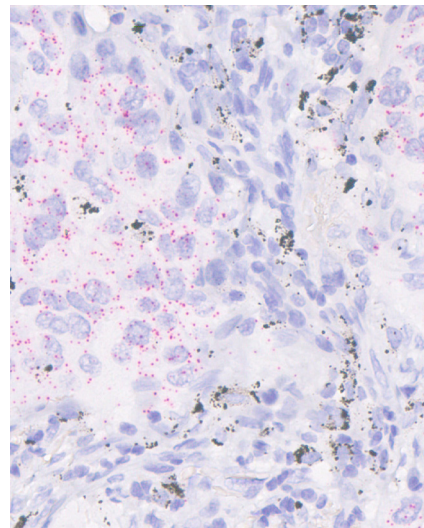


FIGURE 3. Visualization of exon junction *MET* E15/E16 expression in human lung cancer using BaseScope VS Reagent Kit and the DISCOVERY ULTRA platform from Roche Tissue Diagnostics.

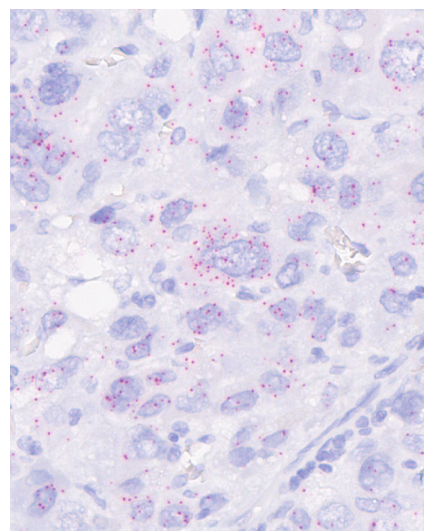


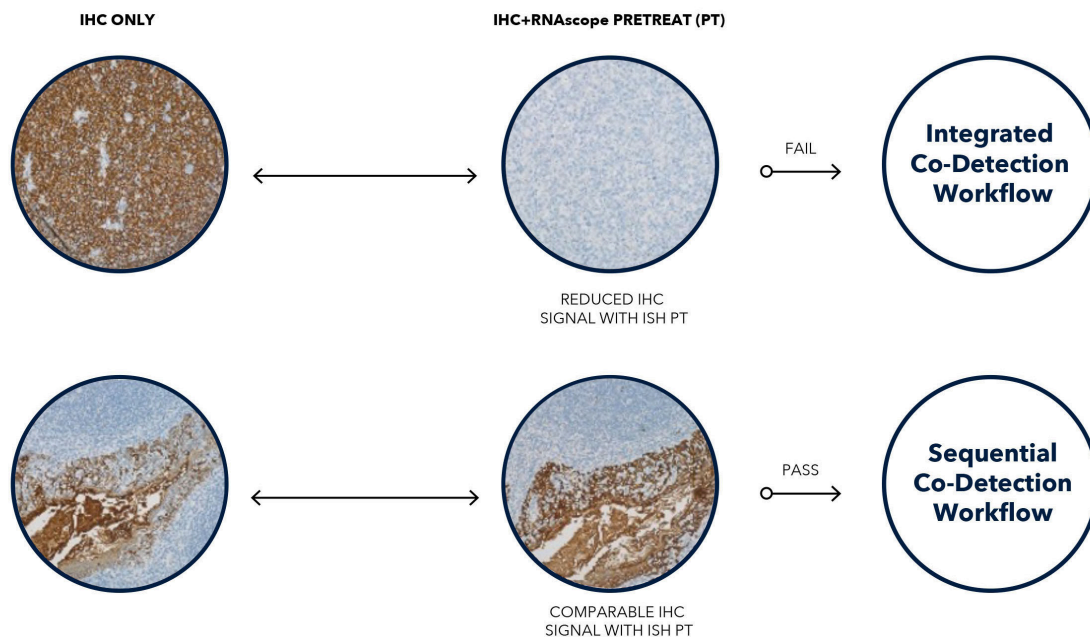
FIGURE 4. Visualization of exon junction *EGFR* E7/E8 expression in human liver cancer using BaseScope VS Kit-Red and DISCOVERY ULTRA platform from Roche Tissue Diagnostics.



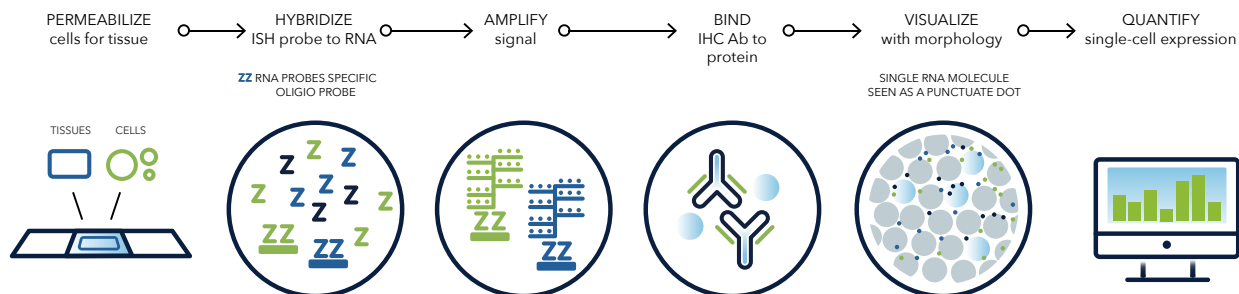
# RNA-Protein Co-detection workflow and reagents allows researchers to perform ISH and IHC on the same sample in the same experiment

RNA-Protein Co-Detection reagents and workflow allows researchers to perform *in situ* hybridization (ISH) and immunohistochemistry (IHC) on the same sample in the same experiment.

To explore the complete tool set we provide for your research success ,we recommend starting with your IHC validated antibody of choice and running it with the RNAscope Pretreatment , this will serve as a guideline for selecting one of the workflows as shown in the example below



## SEQUENTIAL



# INTEGRATED

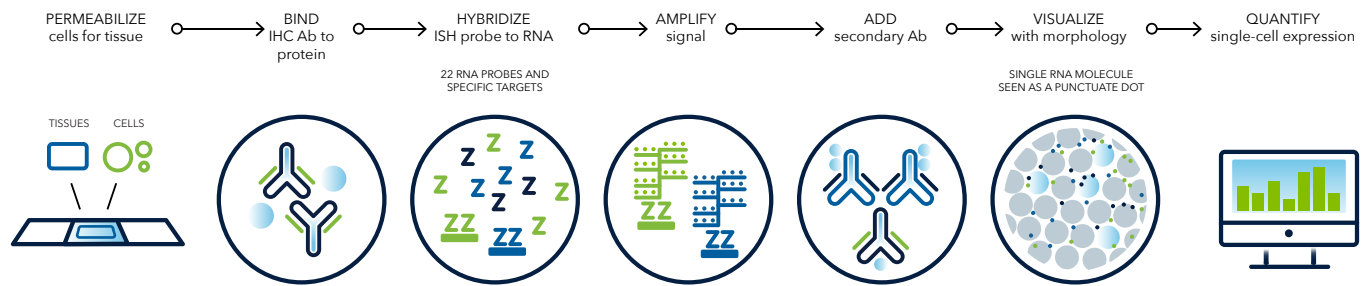


FIGURE 5. The new integrated and sequential Co-detection workflow for simultaneous RNA and protein detection

These workflows automate detection of RNA and Protein using the Roche Discovery ULTRA platform. Being able to automate IHC and ISH on the same slide reduces the amount of precious sample consumed when research goals require both protein and RNA expression data. These workflows and reagents will allow inclusion of wide range of antibodies, including those targeting protease-sensitive epitopes.

Both workflows utilize ACD's patented RNAscope and BaseScope signal amplification and background suppression technologies to deliver supreme specificity and sensitivity with optimal signal-to-noise ratio.

The new VS RNA-Protein Co-Detection assays also enable simultaneous unbiased detection of cell type marker and secreted proteins. The assays address scientific questions such as complexities of splice variants and biomarker levels in cancer biology.

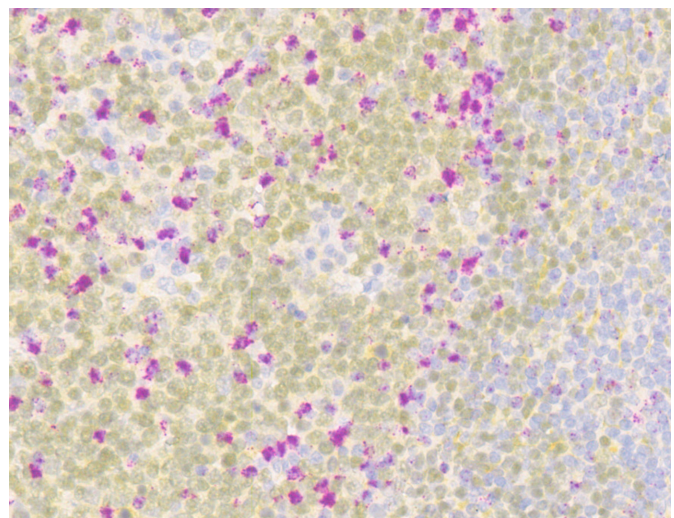


FIGURE 6. PAX5 protein visualized with *PDCD1* in Tonsil. F

Combined detection of a gene's RNA and protein in the same sample supports key applications that include:

- Detect pathogens and host cell markers
- Detect non-coding RNA in target cells
- Identify cellular source of secreted proteins
- Enable detection of additional targets such as splice variants, highly homologous transcripts in specific cell types
- Visualize cell margin and RNA targets
- Correlate RNA-protein expression
- Validate antibody specificity

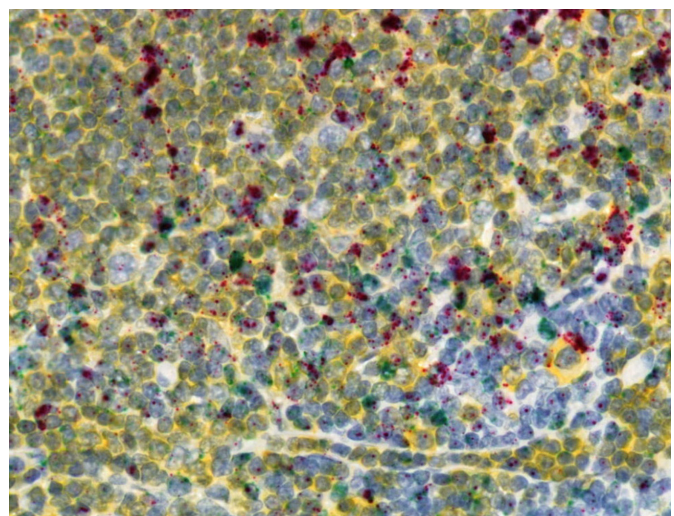


























FIGURE 7. CD20 protein visualized with *CTLA4* and *PDCD1* in Tonsil.

# Product Ordering Information

## CHROMOGEN COMBINATIONS FOR ICW ON DISCOVERY ULTRA

For available options for ISH – IHC chromogen combinations,  
see the following table:



ACD ISH Assay	Reagents for ISH Detection		IHC Detection System/Reagents	
VS Universal RNAscope AP (chromogenic)		RNAscope VS Universal AP Reagent Kit and mRNA Red Detection Kit		DISCOVERY AP-conjugated secondary with DISCOVERY Yellow (AP) IHC chromogen
				DISCOVERY HRP-conjugated secondary with DISCOVERY Teal HRP
				DISCOVERY HRP-conjugated secondary with DISCOVERY Green HRP
VS Universal RNAscope HRP (chromogenic)	   	RNAscope VS Universal HRP Reagent Kit		DISCOVERY AP-conjugated secondary with DISCOVERY Yellow (AP) IHC Chromogen
		mRNA Green Detection Kit with DISCOVERY Inhibitor		DISCOVERY AP-conjugated secondary with ChromoMap Red (AP)
		mRNA DAB Detection Kit		DISCOVERY HRP-conjugated secondary with DISCOVERY Teal HRP
		mRNA Purple Detection Kit with DISCOVERY Inhibitor		DISCOVERY HRP-conjugated secondary with DISCOVERY Green HRP
		mRNA Teal Detection Kit with DISCOVERY Inhibitor		DISCOVERY HRP-conjugated secondary with DISCOVERY Purple HRP
VS Universal RNAscope HRP (fluorescent)		RNAscope VS Universal HRP Reagent Kit DISCOVERY Fluorescent Detection Kit with DISCOVERY Inhibitor		DISCOVERY HRP-conjugated secondary
VS Universal RNAscope Duplex (chromogenic)	   	RNAscope VS Duplex Reagent Kit		DISCOVERY AP-conjugated secondary with DISCOVERY Yellow (AP) IHC Chromogen
		mRNA Red Detection Kit		
		mRNA Green Detection Kit		DISCOVERY HRP-conjugated secondary with DISCOVERY Teal HRP
		mRNA Teal Detection Kit mRNA DAB Detection Kit		DISCOVERY HRP-conjugated secondary with DISCOVERY Green HRP
VS BaseScope (AP) (chromogenic)		BaseScope VS Universal AP Reagent Kit mRNA Red Detection Kit		DISCOVERY AP-conjugated secondary with DISCOVERY Yellow (AP) IHC chromogen
				DISCOVERY HRP-conjugated secondary with DISCOVERY Teal HRP
				DISCOVERY HRP-conjugated secondary with DISCOVERY Green HRP

## RNAscope REAGENTS

RNAscope VS Universal AP / HRP Reagents: Order from Advanced Cell Diagnostics		
Product Code		Product Name
General Reagents (Required for VS Universal HRP and AP)	323740	RNAscope VS Sample Prep Reagent Kit v2
	320630	RNAscope VS Accessory Kit
VS Universal HRP Reagents	323210	RNAscope VS Universal HRP Detection Reagents
VS Universal AP Reagents	323260	RNAscope VS Universal AP Detection Reagents

## RNAscope REAGENTS

RNAscope VS Universal AP / HRP Reagents: Order from Roche		
Component		Ordering Code
VS Universal HRP Reagents	mRNA Probe Amplification Kit - fill dispensers with VS Universal HRP AMP 1-7	06614337001
	mRNA DAB Detection Kit*	06614353001
	DISCOVERY Inhibitor**	07017944001
	mRNA Purple Detection Kit*	08127166001
	mRNA Green Detection Kit*	08952612001
	mRNA Teal Detection Kit*	08352941001
VS Universal AP Reagents	mRNA RED Probe Amplification Kit - fill dispensers with VS Universal AP AMP 1-7	07095341001
	mRNA RED Detection Kit	07099037001

\* Choose one mRNA Detection Kit for RNAscope HRP detection

\*\* DISCOVERY Inhibitor is not required if using mRNA DAB Detection Kit

RNAscope VS Universal Duplex Reagents: Order from Advanced Cell Diagnostics	
Product Code	Product Name
323310	RNAscope VS Duplex Detection Reagents

RNAscope VS Universal Duplex Reagents (Additional Reagents): Order from Roche	
Component	Ordering Code
mRNA Duplex Amp Kit	08127174001
mRNA Link	08127115001
mRNA RED Detection Kit	07099037001
mRNA DAB Detection Kit*	06614353001
mRNA Green Detection Kit*	08952612001
mRNA Teal Detection Kit*	08352941001

\* Choose one mRNA Detection Kit for VS Duplex Channel 1 detection.

## BaseScope REAGENTS

Order from Advanced Cell Diagnostics	
Product Code	Product Name
323710	BaseScope VS Detection Reagents

Order from Roche	
Component	Product Name
mRNA RED Probe Amplification Kit - fill dispensers with VS Universal AP AMP 1-7	07095341001
mRNA RED Detection Kit	07099037001
Option 8 dispenser	05271916001

## RNA-PROTEIN CO-DETECTION REAGENTS

Order from Advanced Cell Diagnostics	
Product Code	Product Name
323760	VS RNA-Protein Ancillary Reagent Kit

User Supplied Reagents	
Reagent	Quantity
Primary Antibody Concentrate 10% Neutral Buffered Formalin	As needed 5-10 mL

Order from Roche	
Component	Product Name
Probe Dispensers	Please contact Roche
mRNA Sample Prep Kit	08127166001
Antibody Dispensers	Please contact Roche
Pretreatment 4 Dispenser	05280125001
Fixative 1 Dispenser	05271614001
Enzyme 1 Dispenser	05271517001
Counterstain 1 dispenser	05271720001
Counterstain 2 dispenser	05271738001
DISCOVERY Antibody Block	05268869001

Please refer to the Tech Note for additional reagent requirements for ISH Assay and IHC Detection.



# Where Science Intersects Innovation™

**Bio-Techne®** | R&D Systems™ Novus Biologicals™ Tocris Bioscience™ ProteinSimple™ ACD™ ExosomeDx™ Asuragen®



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