



Working together for spatial RNA and protein detection on the DISCOVERY ULTRA instrument

RNAscope® and BaseScope™ ISH Assays from Advanced Cell Diagnostics fully automated on the Roche DISCOVERY ULTRA Platform

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Automate ISH probes from ACD on the VENTANA DISCOVERY ULTRA system

RNAscope® and BaseScope™ probes can be purchased from ACD, and used in combination with VENTANA chromogens, fluorophores, and bulk reagents for fully automated assays on the DISCOVERY ULTRA system.



Detect target RNA expression by

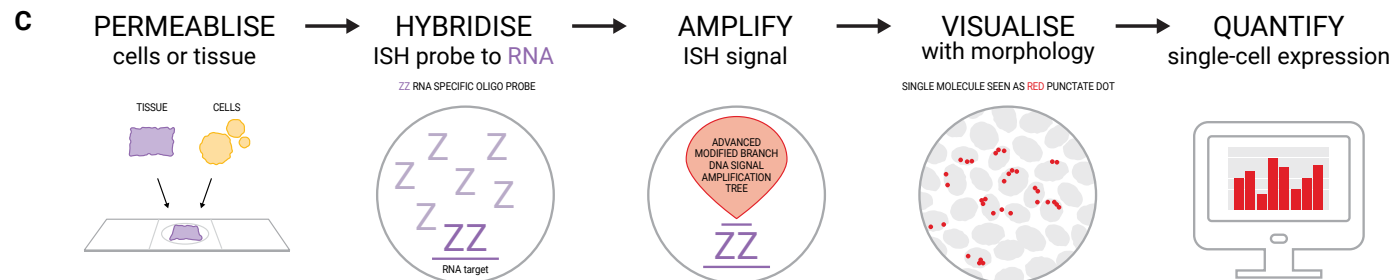
ANY gene | ANY genome | ANY tissue/cell type with *in situ* hybridization

What's the difference?

RNAscope® VS assays are the reference standard in spatial molecular pathology for the widest range of samples. The assays are ideal for co-localisation studies mapping the RNA expression of two targets in the same cell.

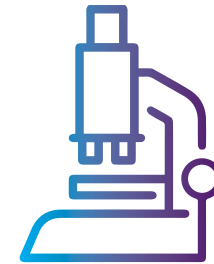
BaseScope™ VS assays are based on proven RNA technology and are ideal for the study of exon junctions and splice variants, detecting the presence of small insertions or deletions within transcripts, and determining relationships among complex, highly homologous gene families.

How do ACD assays work?



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

Choose the optimal chromogen or fluorophore for your specific application



Don't be limited by your detection choices. Roche researchers have developed a range of new chromogens and a spectrum of fluorophores specially formulated to work with ACD probes. These detection options enable you to explore mRNA expression on a wider range of tissue types. Now you can easily see expression in pigmented tissues.

Multiple ways to multiplex

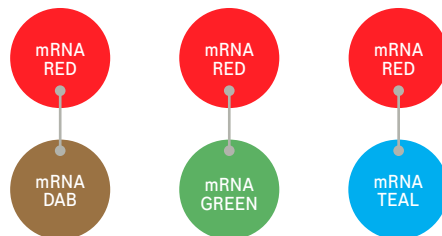
Fluorophores and translucent chromogens enable multiplexing studies in BOTH fluorescent and brightfield applications. Translucent chromogens enable co-localisation studies in brightfield.

mRNA applications - brightfield assays



OR

RNAscope™ ISH (duplex targets)

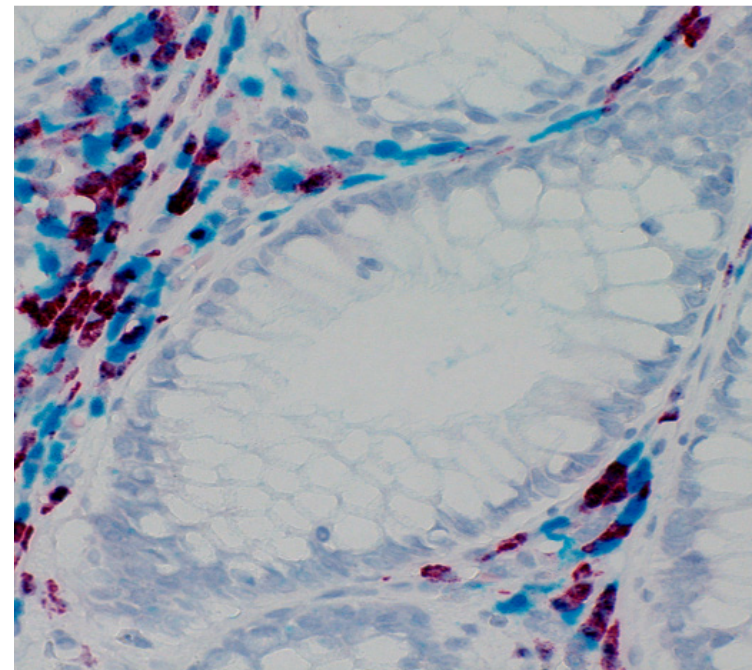


OR

BaseScope™ ISH (single target)



Up to 3 IHC targets

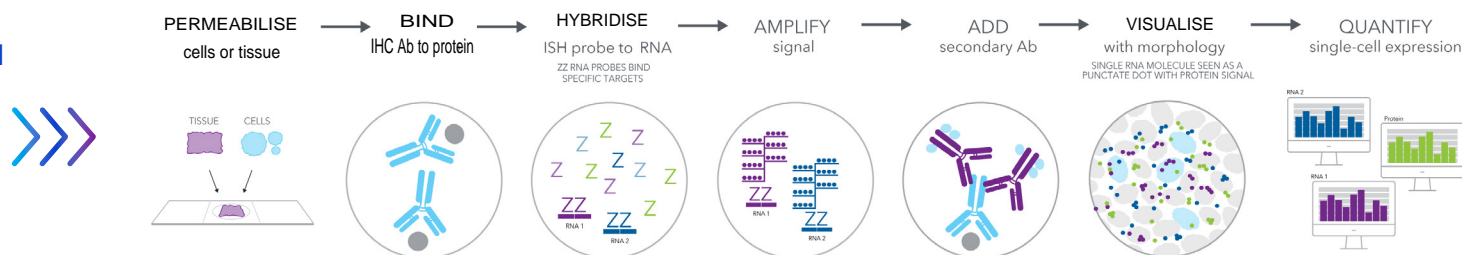


IgL mRNA probe stained with mRNA Red Detection kit and IgK mRNA probe stained with mRNA Teal Detection kit

Combine IHC with ISH using either chromogens or fluorophores

ACD's RNA-protein VS Co-Detection Kit, when used with RNAscope and BaseScope Assays, determines the relationship between mRNA targets and protein expression—on the same slide.

The new Integrated Co-detection Workflow for simultaneous RNA and protein detection



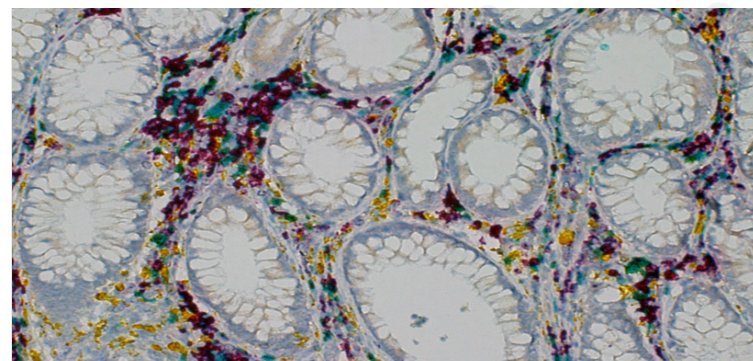
How does it work?

The Integrated Co-Detection Workflow (ICW) automates ACD's RNA & protein co-detection workflow on the Roche DISCOVERY ULTRA platform. IHC and ISH same slide co-detection reduces the amount of precious sample consumed when research goals require both protein and RNA expression data. Combining ISH with IHC provides more data from a single sample.

ICW enables the use of a wide range of antibodies, including those targeting protease-sensitive epitopes.

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

- Detect pathogens and host cell markers
- Confirm RNA expression in cell types using protein biomarkers
- Identify cellular source of secreted proteins
- Visualise cell margin and RNA targets
- Correlate RNA-protein expression

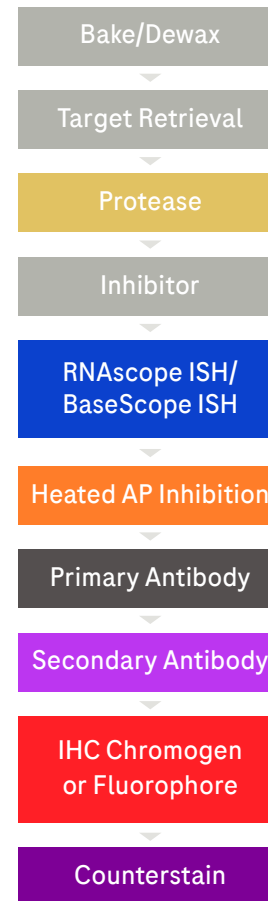


TNFSF9 mRNA probe stained with mRNA Red Detection kit, CD274 mRNA probe stained with mRNA Teal Detection kit, CD8 protein stained with Yellow Detection kit

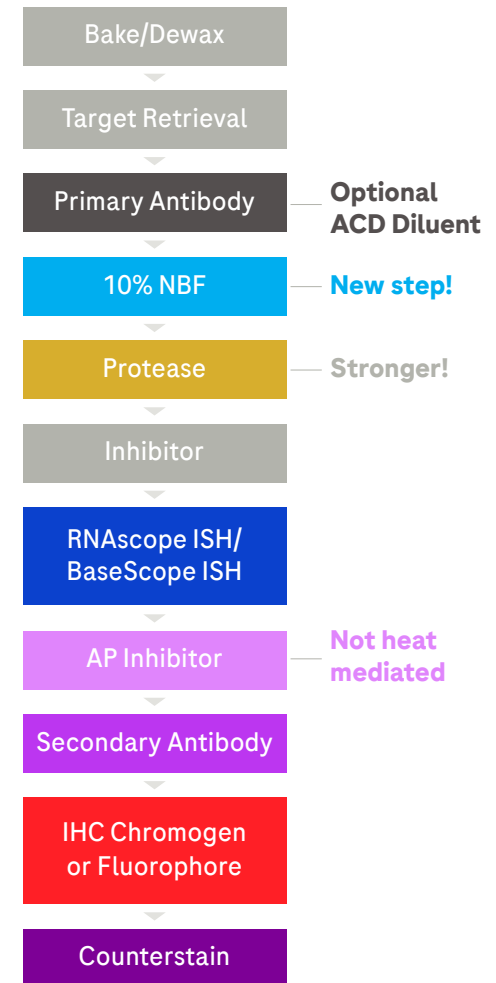
Two workflow options for ISH & IHC multiplexing

For RNAscope ISH to be successfully combined on the same slide with IHC, good pre-analytic techniques must be followed for high quality of both the RNA and protein targets. The Sequential ISH-IHC Workflow, which includes mRNA ISH detection followed by IHC for protein detection, is a straightforward approach to combining these two techniques. However, some protein epitopes may be sensitive to the protease step in the sequential workflow, resulting in weaker or absent IHC staining. To address this issue, the Integrated Co-Detection Workflow (ICW) takes a different approach splitting the IHC assay into two parts. In short, the primary antibody is incubated for binding before the protease step, which then proceeds to ISH staining. The secondary antibodies and detection are then applied after the ISH steps. This strategy may improve the performance of IHC for protease-sensitive epitopes. These two approaches expand the overall success rate of same-slide multiplexed ISH and IHC.

Sequential ISH-IHC



Integrated Co-Detection Workflow (ICW)



ISH & IHC multiplexing detection options



Identifying the chromogens and fluorophores that can be combined for your assay design.

BaseScope	Detection options for ISH			Multiplex 1 or 2 ISH with up to 3 IHC	
	RNAscope single ISH chromogens	RNAscope single ISH fluorophores	RNAscope duplex ISH chromogens	IHC chromogens	IHC fluorophores
mRNA Red	mRNA Red	DISCOVERY FITC		ChromoMAP DAB	DISCOVERY FITC
	mRNA DAB	DISCOVERY FAM	mRNA Red/mRNA DAB	DISCOVERY Red	DISCOVERY FAM
	mRNA Teal	DISCOVERY Rhodamine	mRNA Red/mRNA Teal	ChromoMAP Red	DISCOVERY Rhodamine
	mRNA Green	DISCOVERY Red 610	mRNA Red/mRNA Green	DISCOVERY Teal HRP	DISCOVERY Red 610
	mRNA Purple	DISCOVERY Rhodamine 6G		DISCOVERY Green HRP	DISCOVERY Rhodamine 6G
		DISCOVERY DCC		DISCOVERY Purple	DISCOVERY DCC
		DISCOVERY Cy5		ChromoMAP Blue	DISCOVERY Cy5
				DISCOVERY Yellow	
				DISCOVERY Silver	



Order these materials from ACD for **ALL** assay types

- RNAscope® 2.5 VS Target Probes
- RNAscope® 2.5 VS Control Probes
- RNAscope® Control Slides (optional)



Order these materials from Roche for **ALL** assay types

- 250 Test Probe Dispenser (1-25)—order one for each different probe
- DISCOVERY mRNA Sample Prep kit

Based on your experiment type, order these additional components

STEP 1: Choose your experiment	RNAscope® chromogenic singleplex	RNAscope® chromogenic duplex	BaseScope™ chromogenic singleplex	RNAscope® fluorescent singleplex	Co-detection (IHC + ISH) experiments
STEP 2: Order these reagents from ACD	For DAB, green, purple or teal, order: RNAscope® VS Universal HRP Reagent kit For red, order: RNAscope® VS Universal AP Reagent kit	RNAscope® VS Duplex Reagent kit	BaseScope™ VS Reagent kit	RNAscope® VS Universal HRP Reagent kit	VS RNA-Protein Co-detection Ancillary Kit For DAB, green, purple or teal, order: RNAscope® VS Universal HRP Reagent kit For red, order: RNAscope® VS Universal AP Reagent kit
STEP 3: Order these reagents from Roche	For DAB, green, purple or teal, order: DISCOVERY mRNA Probe AMP kit DISCOVERY Inhibitor (not needed for DAB) For red, order: DISCOVERY mRNA Red Probe AMP kit	DISCOVERY mRNA Duplex Amplification kit DISCOVERY mRNA Link DISCOVERY Inhibitor (not needed for DAB)	DISCOVERY mRNA Red Probe AMP kit 250 Test Option 8 dispenser	DISCOVERY mRNA Probe AMP kit DISCOVERY Inhibitor	Pre-treatment 4 Dispenser Fixative 1 Dispenser Enzyme 1 Dispenser DISCOVERY Antibody Block Appropriate mRNA AMP kit for chromogen/fluorophore selected
STEP 4: Choose your chromogens or fluorophores from Roche	DISCOVERY mRNA Green HRP detection kit DISCOVERY mRNA Teal HRP detection kit DISCOVERY mRNA Purple HRP detection kit DISCOVERY mRNA DAB detection kit DISCOVERY mRNA Red detection kit	DISCOVERY mRNA Red detection kit Plus 1 of the following: DISCOVERY mRNA Green HRP detection kit DISCOVERY mRNA Teal HRP detection kit DISCOVERY mRNA DAB detection kit	DISCOVERY mRNA Red detection kit	DISCOVERY CY5 kit DISCOVERY DCC kit DISCOVERY FAM kit DISCOVERY FITC kit DISCOVERY Red 610 DISCOVERY Rhodamine DISCOVERY Rhodamine 6G	Refer to table in ICW section on page 5



Ordering information



ACD reagents	Catalog number	Quantity	Description
VS RNA-Protein Co-detection Ancillary Kit	323760	60 tests	VS Co-detection Antibody Diluent, Protease and Inhibitor; Reagents to fill Roche Enzyme dispenser and Pretreatment dispensers
RNAscope VS Universal HRP Reagent kit	323200	60 tests	Includes RNAscope VS Universal HRP Detection Reagents, RNAscope Universal VS Sample Prep Reagents v2, and RNAscope VS Accessory kit
RNAscope VS Universal AP Reagent kit	323250	60 tests	Includes RNAscope VS Universal AP Detection Reagents, RNAscope Universal VS Sample Prep Reagents v2, and RNAscope VS Accessory kit
RNAscope VS Duplex Reagent kit	323300	60 tests	Includes RNAscope VS Universal Duplex Detection Reagents, RNAscope Universal Sample Prep reagents, RNAscope VS Accessory kit, and RNAscope Target Retrieval Reagents
BaseScope VS Reagent kit	323700	60 tests	Includes BaseScope VS Detection Reagents, RNAscope Universal VS Sample Prep Reagents v2, and RNAscope VS Accessory kit





Roche RNAscope reagents	Part number	Ordering code	Pack size (tests)	Description
mRNA Sample Prep kit	760-248	08127166001	60	User fillable dispensers to be filled with RNAscope VS Sample Prep reagents
mRNA Probe Amplification kit	760-222	06614337001	60	User fillable dispensers to be filled with RNAscope VS Universal HRP Detection reagents
mRNA RED Probe Amplification kit	760-236	07095341001	60	User fillable dispensers to be filled with RNAscope VS Universal AP Detection reagents
mRNA Duplex Amp kit	760-249	08127174001	60	User fillable dispensers to be filled with RNAscope VS Duplex Detection reagents
mRNA Link	760-6014	08127115001	60	Ready to use ancillary reagent for RNAscope Duplex assays
Probe 1-25 Dispenser	960-761 -960-785	See DISCOVERY Catalog	250	User fillable dispensers to be filled with ACD Probes
Option 8 Dispenser	771-758	05271916001	250	User fillable dispensers to be filled with BaseScope VS AMP 8 reagent
Pretreatment 4 Dispenser	960-904	05280125001	250	User fillable dispensers essential for ICW assays; to be filled with VS Co-Detection Inhibitor
Fixative 1 Dispenser	771-731	05271614001	250	User fillable dispensers essential for ICW assays; to be filled with 10% NBF
Enzyme 1 Dispenser	771-721	05271517001	250	User fillable dispensers essential for ICW assays; to be filled with VS Co-Detection Protease
Antibody Dispenser	770-001 -770-150	See DISCOVERY Catalog	250	User fillable dispensers essential for IHC assays; to be filled with primary antibody
Chromogens				
mRNA DAB Detection kit	760-224	06614353001	60	Ready to use chromogenic detection kit
mRNA Purple HRP Detection kit	760-255	08127166001	60	Ready to use chromogenic detection kit
mRNA Teal HRP Detection kit	760-256	08352941001	60	Ready to use chromogenic detection kit
mRNA Green HRP Detection kit	760-278	08952612001	60	Ready to use chromogenic detection kit
mRNA RED Detection kit	760-234	07099037001	60	Ready to use chromogenic detection kit

Fluorophores	Part number	Ordering code	Pack size (tests)	Description
DISCOVERY FITC	760-232	07259212001	50	Ready to use fluorescent detection kit
DISCOVERY Rhodamine	760-233	07259883001	50	Ready to use fluorescent detection kit
DISCOVERY Cy5	760-238	07551215001	50	Ready to use fluorescent detection kit
DISCOVERY Rhodamine 6G	760-244	07988168001	50	Ready to use fluorescent detection kit
DISCOVERY Red 610	760-245	07988176001	50	Ready to use fluorescent detection kit
DISCOVERY FAM	760-243	07988150001	50	Ready to use fluorescent detection kit
DISCOVERY DCC	760-240	07988192001	50	Ready to use fluorescent detection kit
Bulks & ancillaries				
DISCOVERY Wash Buffer	950-510	07311079001	NA	DISCOVERY ULTRA system bulk reagent
ULTRA LCS	650-210	05424534001	NA	DISCOVERY ULTRA system bulk reagent
10x SSC	950-110	05353947001	NA	DISCOVERY ULTRA system bulk reagent
Reaction Buffer (10x)	950-300	05353955001	NA	DISCOVERY ULTRA system bulk reagent
DISCOVERY CC1	950-500	06414575001	NA	DISCOVERY ULTRA system bulk reagent
Hematoxylin II	790-2208	05277965001	250	DISCOVERY ULTRA Counterstain
Bluing Reagent	760-2037	05266769001	250	DISCOVERY ULTRA Counterstain
QD DAPI	760-4198	05268826001	125	DISCOVERY ULTRA fluorescent counterstain
DISCOVERY Inhibitor	760-4840	07017944001	250	Endogenous peroxidase inhibitor for mRNA Purple, Green, Teal or IF HRP Detection kits
DISCOVERY Antibody Block	760-4204	05268869001	250	Blocking reagent for ISH/IHC staining assays
Roche BaseScope reagents				
mRNA Sample Prep kit	760-248	08127166001	60	User fillable dispensers to be filled with RNAscope VS Sample Prep reagents
mRNA RED Probe Amplification kit	760-236	07095341001	60	User fillable dispensers to be filled with BaseScope VS Amp reagents
Probe 1-25 Dispenser	960-761 -960-785	See DISCOVERY Catalog	250	User fillable dispensers to be filled with ACD Probes
Option 8 Dispenser	771-758	05271916001	250	User fillable dispensers to be filled with BaseScope VS AMP 8 reagent
mRNA RED Detection kit	760-234	07099037001	60	Ready to use chromogenic detection kit