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USER MANUAL

RNAscope® VS Duplex Assay

For Ventana DISCOVERY™ ULTRA System

RED / TEAL AND RED / BROWN

Document Number 323300-USM-ULT

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Citing RNAscope® Assay in Publications

When describing a procedure for publication using this product, please refer to it as the RNAscope® Assay and cite: Wang F, Flanagan J, Su N, Wang L-C, Bui S, Nielson A, Wu X, Vo H-T, Ma X-J and Luo Y. RNAscope®: A Novel *In Situ* RNA Analysis Platform for Formalin-Fixed Paraffin-Embedded Tissues. J. Mol. Diagnostics, 2012, 14:22–29.

Disclaimers

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Contents

1 Chapter 1. Product Information

Before using this product, read and understand the information in **Appendix B. Safety** on page 37 in this document.

IMPORTANT! We recommend reading the entire user manual before beginning any protocols.

About this guide

This user manual provides two versions of the RNAscope® VS Universal Assay:

- **Chapter 4. Automated RNAscope® VS Duplex Assay** starting on page 16.
- **Appendix A. Semi-automated RNAscope® VS Duplex Assay** starting on page 27.

Product description

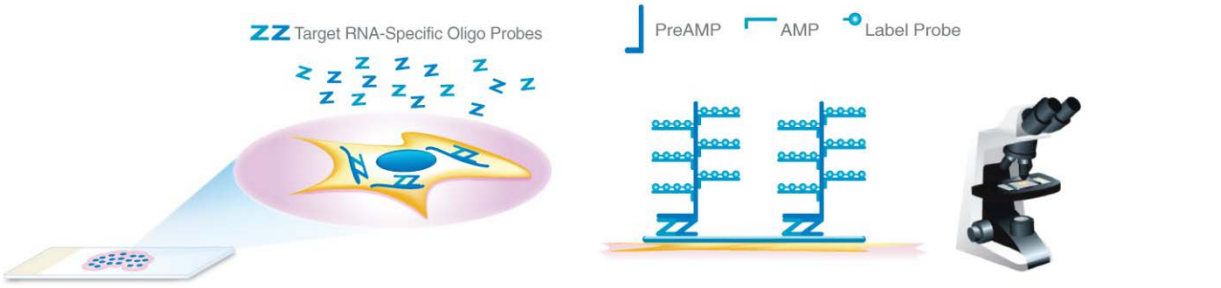
Background

The RNAscope® VS Assays use a novel and proprietary method of *in situ* hybridization (ISH) to visualize single RNA molecules per cell in formalin-fixed, paraffin-embedded (FFPE) tissue mounted on slides. The assays are based on Advanced Cell Diagnostic's patented signal amplification and background suppression technology, and can detect RNA molecules in archival samples and partially degraded specimens. The RNAscope® VS Duplex Assay allows users to automate the highly sensitive RNAscope® Assay using the Ventana DISCOVERY™ ULTRA System.

Overview

Figure 1 on page 7 illustrates the RNAscope® VS Duplex Assay procedure, which can be completed on the instrument in ~14-16 hours. Starting with properly prepared samples, sections are first pretreated, and then RNA-specific probes are hybridized to target RNA. The signal is amplified using multiple steps, followed by hybridization to horseradish peroxidase (HRP)- and alkaline phosphatase (AP)-labeled probes and detection using a chromogenic substrate. Each single RNA transcript appears as a distinct dot of chromogen precipitate visible using a common bright-field microscope.

Figure 1. Procedure overview



1: Tissue section	2: Hybridize to target RNA	3: Amplify signal	4: Image
Start with properly prepared sections and pretreat to allow access to target RNA.	Hybridize gene-specific probe pairs to the target mRNA.	Probes are hybridized to a cascade of signal amplification molecules, culminating in binding of HRP- or AP-labeled probes. The Duplex Assay enhances signal further with additional amplification steps. Add DAB and Fast Red substrates to detect target RNA.	Visualize target RNA using a standard bright field microscope.

Kit contents and storage

The RNAscope® VS Duplex Assay requires the RNAscope® 2.5 VS Probes, VS C2 probes, and the RNAscope® VS Duplex Reagents, available from Advanced Cell Diagnostics.

RNAscope® VS Probes

The RNAscope® VS Probes consist of the user-specified Target Probe and the Positive and Negative Control Probes. Visit **https://acdbio.com/products** to find a gene-specific Target Probe or appropriate Control Probes. Each probe is sufficient for staining ~30 standard slides. The probes have a shelf life of two years from the date of manufacturing when stored as indicated in the following table:

Target Probes					
☑	Reagent	Cat. No.	Content	Quantity	Storage
	RNAscope® 2.5 VS Target Probe – *[species]– [gene]*	Various	Ready-To-Use (RTU) probe for color channel 1	7 mL x 1 bottle	2–8°C
	RNAscope® 2.5 VS Target Probe – *[species]– [gene]*– C2	Various	16x probes for color channel 2	440 µL x 1 tube	2–8°C
Control Probes					
☑	Reagent	Cat. No.	Content	Quantity	Storage
	RNAscope® 2.5 VS Duplex Control Probes – (PPIB-C1, Polr2A-C2)	Various	RTU mixture of two probes targeting PPIB in channel C1 and POLR2A in channel C2.	7 mL x 1 bottle	2–8°C
	RNAscope® 2.5 VS Duplex Negative Control Probe – (DapB-C1, DapB-C2)	320759	RTU mixture negative control probes a bacterial gene in channel C1 and in channel C2.	7 mL x 1 bottle	2–8°C

RNAscope® 2.5 VS Probes

The RNAscope® 2.5 VS Probes consist of the user-specified Target Probe and the Positive and Negative Control Probes. Visit **https://acdbio.com/products** to find a gene-specific Target Probe or appropriate Control Probes.

Each Target Probe contains a mixture of short oligonucleotides designed to bind to a specific target RNA and detectable in one of two color channels, C1 or C2.

Note: Different colors are assigned to the C1 and C2 color channels depending on the particular RNAscope® Assay. The color channels for the RNAscope® VS Duplex Assay are shown in the following table:

Probe Channel ID	Chromogenic Labels	
	Enzyme	Color
C1*	HRP	Dark Brown/ Teal
C2	AP	RED

* Default channel

Channel C1 target probes are Ready-To-Use (RTU), while channel C2 probes are shipped as a 16X concentrated stock. To independently detect two target RNAs in a Duplex assay, each target probe must be in a different color channel C1 and C2.

If you wish to only use the C2 probes, please use "RNAscope® 2.5 VS Blank Probe Diluent" (Cat. No. 300049) to dilute the C2 target probe.

RNAscope® VS Control Slides

The RNAscope® VS Control Slides (Cat. No. 310045 for Human control slide, HeLa; Cat. No. 310023 for Mouse control slide, 3T3) contain FFPE cell pellets sectioned and mounted on slides. The control slides can be used for assay control with the RNAscope® 2.5 VS Duplex Positive Control Probe (PPIB-C1, Polr2A-C2) and RNAscope® 2.5 VS Duplex Negative Control Probe (DapB-C1, DapB-C2). The slides have a shelf life of 9 months from the date of manufacturing when stored at 2–8°C with desiccants.

RNAscope® VS Duplex Reagents

RNAscope® VS Duplex kits provide enough reagents to stain ~60 standard slides. You will receive two kits when you order the RNAscope® VS Duplex Reagent Kit (Cat. No. 323300). RNAscope® VS® Duplex Reagents include:

- RNAscope® VS Duplex Detection Reagents (Cat. No. 323310)
- RNAscope® VS Universal Sample Prep Reagents v2 (Cat. No. 323740)
- RNAscope® VS Accessory Kit (Cat. No. 320630)

The reagents are Ready-To-Use (RTU) and have a shelf life of nine months from the date of manufacturing when stored as indicated in the following table:

RNAscope® VS Duplex Detection Reagents (Cat. No. 323310)				
☑	Cat. No.	Reagent	Quantity	Storage
	323311	RNAscope® VS Duplex AMP 1	14 mL x 1 bottle	2–8°C
	323312	RNAscope® VS Duplex AMP 2	14 mL x 1 bottle	2–8°C
	323313	RNAscope® VS Duplex AMP 3	14 mL x 1 bottle	2–8°C

RNAscope® VS Duplex Detection Reagents (Cat. No. 323310)				
☑	Cat. No.	Reagent	Quantity	Storage
	323314	RNAscope® VS Duplex AMP 4	14 mL x 1 bottle	2–8°C
	323315	RNAscope® VS Duplex AMP 5	14 mL x 1 bottle	2–8°C
	323316	RNAscope® VS Duplex AMP 6	14 mL x 1 bottle	2–8°C
	323317	RNAscope® VS Duplex AMP 7	14 mL x 1 bottle	2–8°C
	323318	RNAscope® VS Duplex AMP 8	14 mL x 1 bottle	2–8°C
	323319	RNAscope® VS Duplex AMP 9	14 mL x 1 bottle	2–8°C
	323320	RNAscope® VS Duplex AMP Wash	14 mL x 2 bottles	2–8°C
	323218	RNAscope® VS Protease	14 mL x 1 bottle	2–8°C
RNAscope® VS Universal Sample Prep Reagents v2 Kit (Cat. No. 323740)				
☑	Cat. No.	Reagent	Quantity	Storage
	323741	RNAscope® VS Universal Target Retrieval v2	10 mL x 2 bottles	Room Temp (15–30°C)
	323742	RNAscope® VS Unievrsal Dewax	14 mL x 1 bottle	Room Temp (15–30°C)
RNAscope® VS Acessory Kit (Cat. No. 320630)				
☑	Cat. No.	Reagent	Quantity	Storage
	320631	RNAscope® VS Hematoxylin — RTU	7 mL x 1 bottle	2–8°C
	320632	RNAscope® VS Bluing Reagent — RTU	7 mL x 1 bottle	2–8°C

IMPORTANT! Dewax must be in solution and at room temperature before use on the instrument. Warm in the hand, or place at **37°C** for **15 MIN** before each use before each use regardless of the prior storage condition, since it may precipitate during shipment.

IMPORTANT! Use only RNAscope® 2.5 VS Probes. Do not substitute the reagent components of the RNAscope® VS Duplex Reagent Kit with those of other RNAscope® Reagent Kits, including RNAscope® VS Duplex Reagent Kits or those having the same name.

Required materials from Roche Diagnostics

The RNAscope® VS Duplex Assay requires specific materials and equipment available *only* from Roche Diagnostics (Ventana Medical Systems, Inc.). Catalog Numbers are valid in the U.S. only. For other regions, please check Catalog or ordering numbers with your local lab supplier.

Probe Dispensers (Cat. No. 960-761 to 960-785; for Ordering Code, please contact local Roche representative)		
☑	Component	Storage
	250 Test Probe #1–20 dispensers — fill dispensers with RNAscope® 2.5 VS Probes. Use up to 20 probes at a time.	Room Temp (15–30°C)
mRNA Sample Prep Kit (Cat. No. 760-248; Ordering Code 08127166001)		
☑	Component	Storage
	mRNA Target Retrieval dispenser — fill dispenser with RNAscope® VS Universal Target Retrieval v2	Room Temp (15–30°C)
	mRNA Dewax — fill dispenser with RNAscope® VS Universal Dewax	Room Temp (15–30°C)
	mRNA Protease dispenser — fill dispenser with RNAscope® VS Protease	Room Temp (15–30°C)
mRNA Duplex Amp Kit (Cat. No. 760-249; Ordering Code 08127174001)		
☑	Component	Storage
	mRNA AMP 1 dispenser — fill dispenser with VS Duplex AMP 1	Room Temp (15–30°C)
	mRNA AMP 2 dispenser — fill dispenser with VS Duplex AMP 2	Room Temp (15–30°C)
	mRNA AMP 3 dispenser — fill dispenser with VS Duplex AMP 3	Room Temp (15–30°C)
	mRNA AMP 4 dispenser — fill dispenser with VS Duplex AMP 4	Room Temp (15–30°C)
	mRNA AMP 5 dispenser — fill dispenser with VS Duplex AMP 5	Room Temp (15–30°C)
	mRNA AMP 6 dispenser — fill dispenser with VS Duplex AMP 6	Room Temp (15–30°C)
	mRNA AMP 7 dispenser — fill dispenser with VS Duplex AMP 7	Room Temp (15–30°C)
	mRNA AMP 8 dispenser — fill dispenser with VS Duplex AMP 8	Room Temp (15–30°C)
	mRNA AMP 9 dispenser — fill dispenser with VS Duplex AMP 9	Room Temp (15–30°C)
	mRNA AMP Wash dispenser — fill dispenser withVS Duplex AMP Wash	Room Temp (15–30°C)
mRNA RED Detection Kit (Cat. No. 760-234; Ordering Code 7099037001)		
☑	Component	Storage
	mRNA Inhibitor-prefilled	2–8°C
	mRNA Activator dispenser-prefilled	2–8°C
	mRNA Napthol dispenser-prefilled	2–8°C
	mRNA Fast Red dispenser-prefilled	2–8°C
Generic Dispensers (Cat. No. 771-741; Ordering Code 05271720001, Cat. No. 771-742; Ordering Code 5271738001)		
☑	Component	Storage
	250 Test Counterstain 1 dispenser — fill dispenser with VS Hematoxylin	Room Temp (15–30°C)
	250 Test Counterstain 2 dispenser — fill dispenser with Bluing Reagent	Room Temp (15–30°C)
mRNA DAB Detection Kit (Cat. No. 760-224; Ordering Code 06614353001)		
☑	Component	Storage
	mRNA Inhibitor-prefilled	2–8°C
	mRNA DAB dispenser-prefilled	2–8°C
	mRNA H_2O_2 dispenser-prefilled	2–8°C
	mRNA Copper dispenser-prefilled	2–8°C

mRNA Teal Detection Kit (Cat. No. 760-256; Ordering Code 08352941001)		
☑	Component	Storage
	mRNA Teal Substrate dispenser-prefilled	2–8°C
	mRNA Teal H_2O_2 dispenser-prefilled	2–8°C
	mRNA Teal Activator dispenser-prefilled	2–8°C
mRNA Link (Cat. No. 760-6014; Ordering Code 08127115001)		
☑	Component	Storage
	mRNA Link	2–8°C

Equipment and buffers

☑	Component	Cat. No./ Ordering Code
	10x DISCOVERY Wash (RUO)	950-510 / 07311079001
	ULTRA LCS (Predilute)	650-210 / 05424534001
	SSC Buffer (10X)	950-110 / 05353947001
	Reaction Buffer (10X)	950-300 / 05353955001
	DISCOVERY CC1	950-500 / 06414575001

IMPORTANT! To run VS Duplex assay successfully, use DISCOVERY Wash (950-510) and not DISCOVERY EZ Prep. In the SSC bulk container, use 2X SSC (950-110) and not Ribowash. You may fill the option bulk container with reaction buffer.

User-supplied materials

IMPORTANT! Do not substitute other materials for the SuperFrost® Plus Slides listed in the following table.

☑	Description	Supplier	Cat. No.
	SuperFrost® Plus Slides (required)	Fisher Scientific	12-550-15
	100% ethanol (EtOH)	American Master Tech Scientific/MLS*	ALREAGAL
	xylene	Fisher Scientific/MLS	X3P-1GAL
	10% neutral-buffered formalin (NBF)	MLS	—
	Paraffin wax	MLS	—
	1X PBS	MLS	—
	Microtome	MLS	—
	Drying oven, capable of holding temperature at 60 +/– 1°C	MLS	—
	EcoMount	Biocare	EM897L
	Tissue-Tek® Vertical 24 Slide Rack	American Master Tech Scientific/MLS	LWSRA24
	Tissue-Tek® Staining Dish (6 required)	American Master Tech Scientific/MLS	LWT4457EA

☑	Description	Supplier	Cat. No.
	Tissue-Tek® Clearing Agent Dish, xylene resistant (4 required)	American Master Tech Scientific/MLS	LWT4456EA
	Cover Glass 24 x 50 mm	Fisher Scientific/MLS	12--545-F
	Distilled water	MLS	—
	Dawn detergent or similar detergent	MLS	—
	Fume hood	MLS	—
	Optional: Glass beaker (1 or 2 L)	MLS	—
	Optional: Hot plate	Fisher Scientific/MLS	11-300-49SHP

* Major Laboratory Supplier in North America. For other regions, please check Catalog Numbers with your local lab supplier.

2 Chapter 2. Before You Begin

Prior to running the RNAscope® VS Duplex Assay on your samples for the first time, we recommend that you:

- Be familiar with the Ventana™ DISCOVERY™ ULTRA system. Refer to the Ventana™ System User Manual.
- Run the assay on FFPE RNAscope® VS Control Slides (Cat. No. 310045 for Human control slide, HeLa; Catalog No. 310023 for Mouse control slide, 3T3) using the Positive and Negative Control Probes.

Important procedural guidelines

- Start with properly fixed and prepared sections. Refer to **Chapter 3. Prepare and Pretreat Samples** on page 14, **Recommended guidelines** on page 24, and to our sample preparation and pretreatment user guides available at **https:// acdbio.com/technical-support/user-manuals**.
- Regularly maintain and clean your automated staining instrument.
- Always run positive and negative control probes on your sample to assess sample RNA quality and optimal permeabilization.
- Do *not* substitute required materials. Assay has been validated with these materials only.
- Follow the protocol exactly for best results.
- Do *not* let your sections dry out during the procedure unless specified in the protocol.
- Use good laboratory practices and follow all necessary safety procedures. Refer to **Appendix B. Safety** on page 37 in this document for more information.

3 Chapter 3. Prepare and Pretreat Samples

Formalin-fixed, paraffin-embedded (FFPE) sample preparation and pretreatment are described in the following protocols.

IMPORTANT! We highly recommend following these guidelines. We cannot guarantee assay results with other preparation methods.

For samples treated differently from the following protocol, please see the sample pretreatment optimization procedure described in **Recommended guidelines** on page 24, and to **https://acdbio.com/technical-support/solutions**.

Prepare FFPE sections

Materials required

- 10% neutral buffered formalin (NBF)
- 1X PBS
- Paraffin wax
- 100% ethanol (EtOH)
- Xylene
- Microtome
- Water bath
- SuperFrost® Plus slides

Fix the sample

1. Immediately following dissection, fix tissue in 10% NBF for **16–32 HRS** at **ROOM TEMPERATURE (RT)**. Fixation time will vary depending on tissue type and size.

   ⚠ **CAUTION!** Handle biological specimens appropriately.

IMPORTANT! Fixation for **<16 HRS** or **>32 HRS** will impair the performance of the RNAscope® VS Duplex Assay.

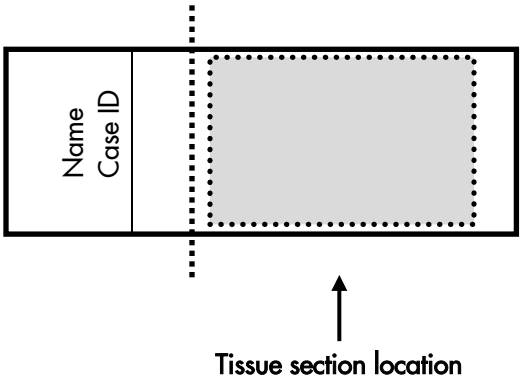
Dehydrate, embed, and cut the sample

IMPORTANT! Use fresh reagents.

1. Wash sample with 1X PBS.
2. Dehydrate sample using a standard ethanol series, followed by xylene.
3. Embed sample in paraffin using standard procedures.

   Note: Embedded samples may be stored at 15–25°C with desiccation. To better preserve RNA quality over a long period (>1 yr), storing at 2–8°C with desiccation is recommended.

4. Trim paraffin blocks as needed, and cut embedded tissue into 5 ± 1 µm sections using a microtome.
5. Place paraffin ribbon in a **40–45°C** water bath, and mount sections on **SUPERFROST® PLUS SLIDES**. Place tissue as shown for optimal staining:



IMPORTANT! Do not mount more than one section per slide. Place sections in the center of the slide.

6. Air dry slides **OVERNIGHT** at **RT**. Do NOT bake slides unless they will be used for RNAscope® within 1 week.

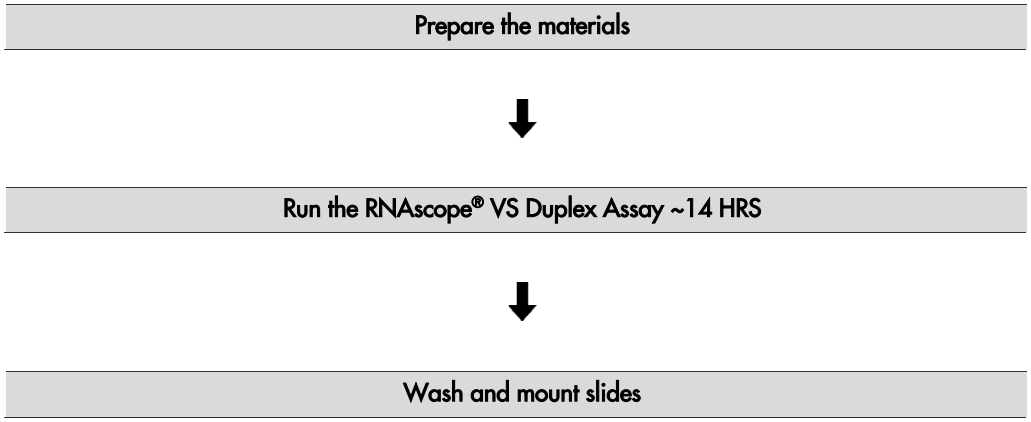
OPTIONAL STOPPING POINT. Use sectioned tissue within 3 months. Store sections with desiccants at **RT**.

4 Chapter 4. Automated RNAscope® VS Duplex Assay

IMPORTANT! We strongly recommend you run the RNAscope® VS Control Slides (Cat. No. 310045 or 310023) using the RNAscope® 2.5 VS positive and negative control probes along with your samples in every run.

Appendix A. Semi-automated RNAscope® VS Duplex Assay describes an offline boiling procedure for use with Cat. No.322000.

Workflow



Prepare the materials

Materials required

Materials Provided by Advanced Cell Diagnostics	Materials Provided by Ventana™ Medical Systems	Other Materials and Equipment
• RNAscope® 2.5 VS Target Probe • RNAscope® 2.5 VS Duplex Positive Control Probe • RNAscope® 2.5 VS Duplex Negative Control Probe • RNAscope® VS Universal Dewax • RNAscope VS Protease • RNAscope® VS Unievrsal Target Retrieval v2 • RNAscope® VS Duplex AMP 1 • RNAscope® VS Duplex AMP 2 • RNAscope® VS Duplex AMP 3 • RNAscope® VS Duplex AMP 4 • RNAscope® VS Duplex AMP 5 • RNAscope® VS Duplex AMP 6 • RNAscope® VS Duplex AMP 7 • RNAscope® VS Duplex AMP 8 • RNAscope® VS Duplex AMP 9 • RNAscope® VS Duplex AMP Wash • RNAscope® VS Hematoxylin • RNAscope® VS Bluing Reagent	• DISCOVERY™ ULTRA — automated slide stainer • DISCOVERY Wash Buffer 10x • ULTRA LCS (Predilute) • SSCBuffer 10X • DISCOVERY CC1 • Reaction Buffer 10X • Probe dispensers • mRNA Sample Prep Kit • mRNA Duplex Amp Kit • mRNA Red Detection Kit • mRNA DAB Detection Kit • mRNA Teal Detection Kit • User fillable dispensers • mRNA Link	• Distilled water • Dawn detergent or similar detergent • Fume hood • xylene • Tissue-Tek® Staining Dish (1) • Tissue-Tek® Clearing Agent Dish, xylene-resistant (1) • Tissue-Tek® Vertical 24 Slide Rack • EcoMount • Cover Glass, 24 mm x 50 mm

Prepare the instrument

If the instrument has not been used for ≥1 week, follow the guidelines for instrument maintenance in the *Ventana™ System User Manual*.

Dilute bulk reagents

Please prepare the bulk fluids as per manufacturer's instructions.

Register new reagents

Reagent dispensers come with appropriate barcode labels and registration buttons for dispensing RNAscope® VS Duplex Reagents. Refer to the *Ventana™ DISCOVERY ULTRA System User Manual* for details. To register reagents:

1. Log all ACD reagents and probes into the software as "log user-fillable reagents" or "'log user-fillable probes".
2. Use the wand that comes with the instrument to register *new* reagent kits.

Prepare instrument reagents

Refer to the table on page 9 to determine the proper dispenser for each reagent.

1. For RNAscope® VS Duplex AMP 1 to AMP 9 and Amp Wash, transfer the entire volume of each component into the correspondingly labeled dispenser.
2. Transfer the remaining RNAscope® VS Duplex Reagents (VS Target Probe, VS Positive Control Probe, VS Negative Control Probe, VS–Universal Dewax, VS Protease, both bottles of VS Universal Target Retrieval v2, VS Hematoxylin, and VS Bluing Reagent) to the correspondingly labeled dispensers.

IMPORTANT! Avoid cross contamination between reagents. Dewax must be warmed to room temperature and be completely in solution before use.

3. Follow the dispenser product insert instructions to properly prime and handle the dispensers.
4. For the duplex target probe:
   a. Determine the volume of duplex probe needed
   b. In a clean container, add the appropriate volume of the 16X C2 probe stock to the desired volume of Ready-to-Use C1 probe. See the table below for some suggestions of volumes:

RTU C1 Volume	C2 Probe Stock Volume
1 mL	60 µL
2 mL	120 µL
3 mL	180 µL
4 mL	240 µL
5 mL	300 µL

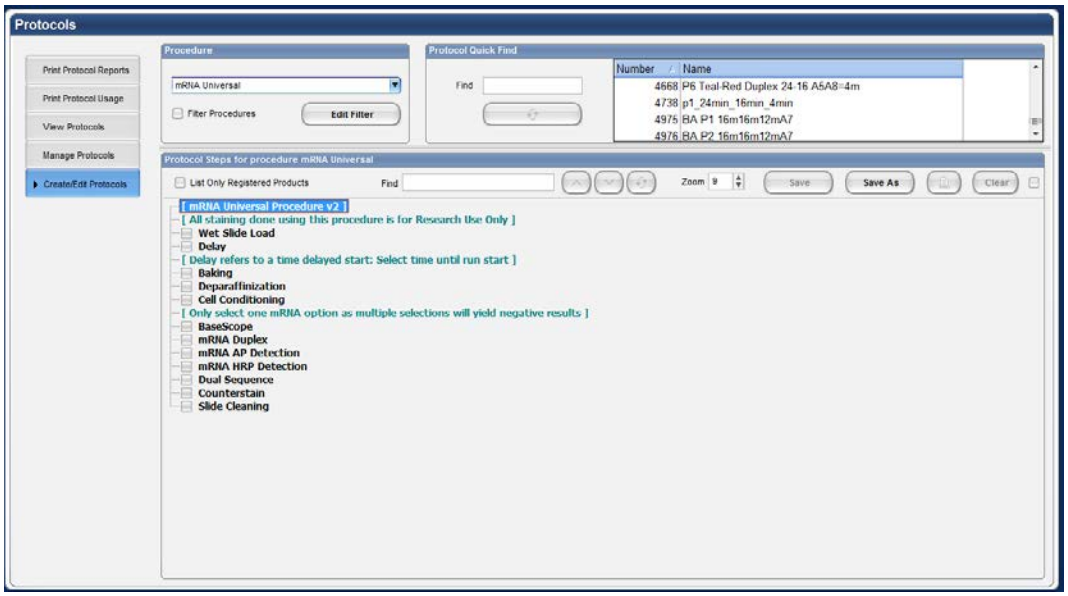
   c. Invert the solution minimally 5 times to mix the probes.
   d. Transfer the solution to a clean Ventana open Probe dispenser.
   e. Properly prime the dispenser and place it on the instrument.
5. Store tightly-capped dispensers (except the Dewax dispenser) at **4°C** when not in use.

IMPORTANT! Do not use expired reagents.

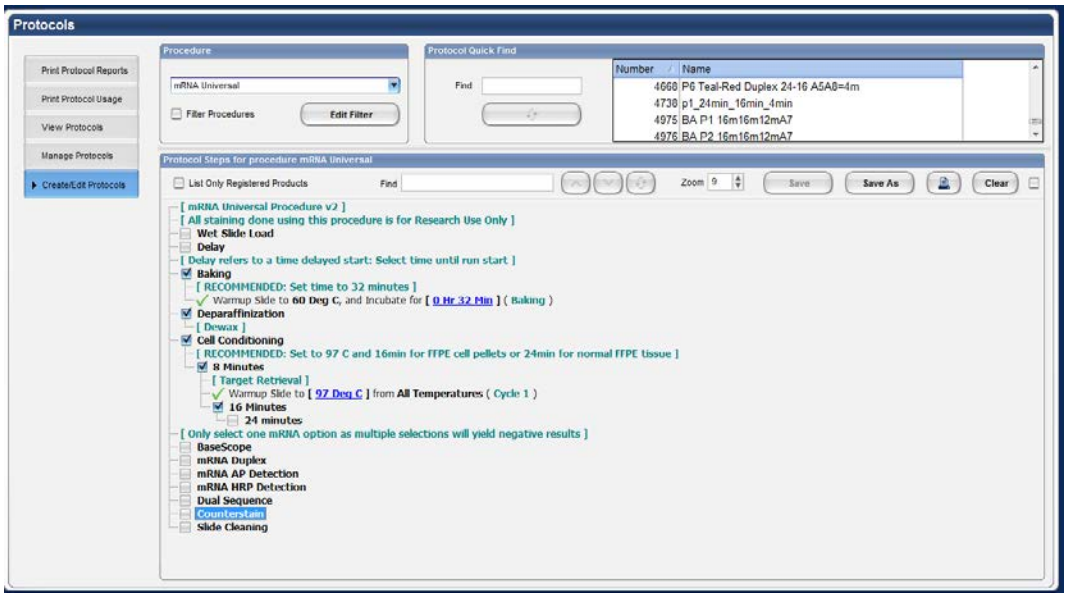
6. Empty the waste bottle if needed.

Create an instrument protocol

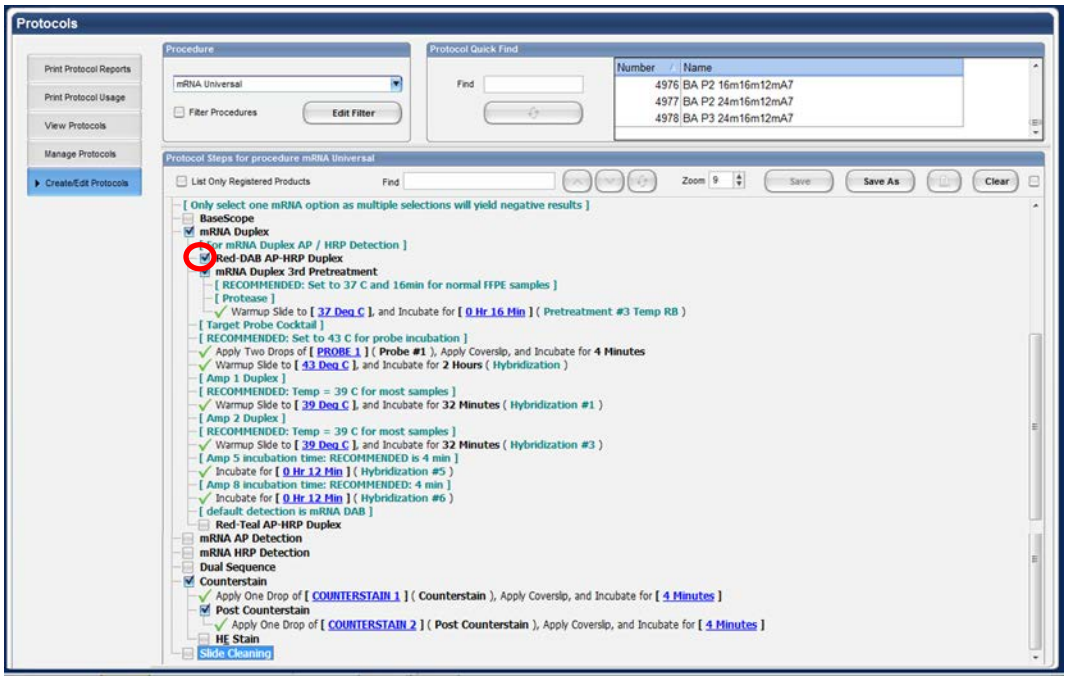
1. Open the VSS software and click on the **Protocol** button.
2. Click on **Create/Edit Protocols**, go to the Procedure drop down menu and select **mRNA Universal**.
3. Main protocol steps appear as shown:



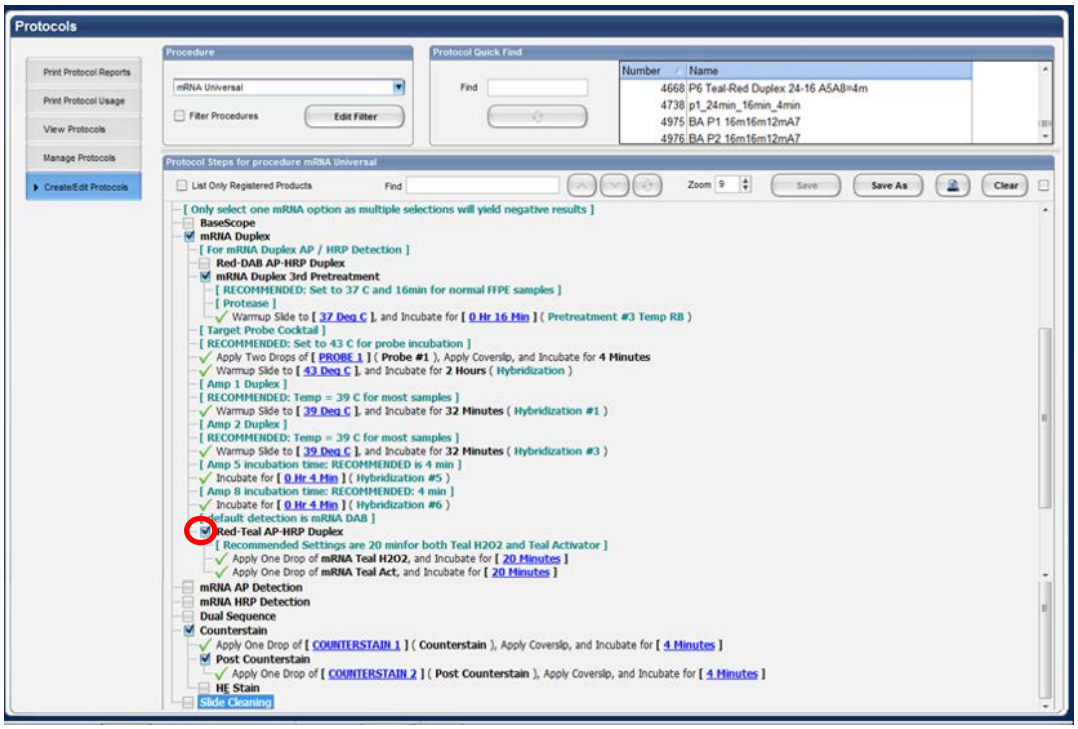
4. After selecting the main protocol steps, drop down menus become available. Select the appropriate protocol steps by clicking on the associated check boxes as shown:



5. (**Red**/**Brown**) After selecting the main protocol steps, drop down menus become available. Note: Make sure to select the Red-DAB AP-HRP Duplex box under the default detection is DAB line. Select the appropriate protocol steps by clicking on the associated check boxes as shown:



6. (**Red**/**Teal**) After selecting the main protocol steps, drop down menus become available. Note: Make sure to select the Red-Teal AP-HRP Duplex box under the *default detection is DAB* line. The suggested times for Teal incubation are **20 min** *mRNA Teal* H_2O_2 and **20 min** *mRNA Teal Act*. Select the appropriate protocol steps by clicking on the associated check boxes as shown:



7. Select the appropriate assay conditions from the drop-down menus according to the following table:

Tissue Type	Cell Conditioning Temperature	Time
Brain	97°C	16 MIN
Cell pellet	97°C	16 MIN
Colon	97°C	24 MIN
Heart	97°C	24 MIN
Intestine	97°C	24 MIN
Kidney	97°C	24 MIN
Liver	97°C	24 MIN
Lung	97°C	24 MIN
Prostate	97°C	24 MIN
Spleen	97°C	24 MIN
Tonsil	97°C	24 MIN

Suggested Temperatures/Times	
VS Protease	37°C
Suggested probe temperatures	Single Probes 43°C
	Pooled Probes 50°C
Suggested Amp 1 and Amp 2 temperatures	39°C
AMP 5 incubation time*	4 MIN
AMP 8 incubation time*	4 MIN

*Staining intensity can be modified by adjusting AMP 5 (Red) and Amp 8 (Brown/Teal) incubation times.

8. Click **Save as**, then select a protocol number from the drop-down menu and choose a protocol name for each probe. Click **Save**.
9. Click **Close** to go back to the main screen.
10. Assign probe number from the list to each probe of interest. For each probe selected, assign a protocol.

Print the labels

1. Select the **Print Label** icon from the upper right corner of the home page screen.
2. Select your preferred template or create a new template. To create a new template, refer to the *Ventana™ DISCOVERY ULTRA System User Manual* for details.
3. Click on **Protocol**.
4. Select the protocol you created for the RNAscope® VS Duplex Assay and click on the **Add** button. When the protocols for all of the slides have been assigned, click on **Close/Print**.
5. Fill in the template for each slide. Select **Print** when completed.
6. Proceed to the following procedure **Load the reagents**.

Run the RNAscope® VS Duplex Assay

Materials required

- Prepared slides
- Prepared instrument reagents
- Prepared detergent
- Distilled water
- Prepared dehydrating materials
- Tissue-Tek® Vertical 24 Slide Rack
- Tissue-Tek® Staining Dish
- EcoMount
- Cover Glass, 24 mm x 50 mm
- Fume hood
- xylene

Load the reagents

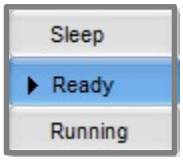
1. Remove the nozzle caps from the filled dispensers and place each cap on the post located on the back of the dispenser.
2. If needed, remove any air bubbles at the nozzle tips by pushing down on the nozzle until the liquid reaches the tip of the nozzle or forms a small meniscus at the tip of the nozzle

IMPORTANT! Do not dispense any drops as this could compromise your drop inventory.

3. Load dispensers onto the reagent racks.
4. Remove the yellow locking ring from the dispensers in the prefilled mRNA RED Detection and mRNA DAB Detection or mRNA Teal Detection Kits. Refer to the instructions provided by Ventana™ Medical Systems.
5. Load the reagent racks onto the reagent carousel.

Start the run

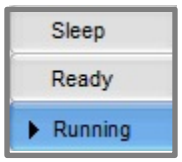
1. Click the **Ready** button.



2. Eject slide drawers.
3. Load each slide onto a heater pad with the label facing upward and inward. Ensure that the slides sit securely on the pads.

IMPORTANT! Prior to loading the slides, ensure heater pads are completely dry. Wipe off any solution using laboratory tissue paper.

4. Close slide drawers.
5. Click the **Running** button. Automated assay will finish in ~**14 HRS**.



IMPORTANT! Before leaving the instrument unattended, ensure all reagents and slides are successfully registered and the instrument is running.

Prepare detergent

1. Prepare 200 mL of diluted detergent by adding 1-2 drops detergent to 200 mL distilled water in a container with a cap.
2. Mix well by inverting the container 4–5 times.
3. Add diluted detergent to a Tissue-Tek® Staining Dish.
   Note: Store diluted detergent at RT.

Prepare dehydrating reagents

- In a fume hood, fill a clearing agent dish with ~200 mL fresh xylene.
  Note: Ensure all containers remain covered when not in use.

Complete the run

1. After the run is complete, remove the mRNA Dewax reagent, place nozzle cap on the dispenser, and store at room temperature.
2. For the remaining reagents, place nozzle caps back on the dispensers and place racks onto magnet locking tray.

IMPORTANT! Store reagent racks at **4°C** until next use. Store the Dewax dispenser at room temperature.

Wash the slides

1. Submerge a Tissue-Tek® Slide Rack into the Tissue-Tek® Staining Dish containing 200 mL diluted detergent.
2. Open the instrument slide drawers and unload slides.
3. Decant solution on the slides into the slide drawer, then *immediately* load slides into the Tissue-Tek® Slide Rack submerged in detergent.
4. Rinse oil off the slides by moving the slide rack up and down in the dish 10 times.
5. Replace the detergent with distilled water and rinse slides by moving the slide rack up and down a minimum of 10 times.
6. Repeat Step 5 three to five times.

Mount the samples

1. Remove the slide rack from the staining dish and dry slides in a **60°C** dry oven for **30 MIN**.

   IMPORTANT! The Red substrate is alcohol sensitive. Do not dehydrate the slides in alcohol.

2. Cool the slides for **5 MIN** at **RT**.
3. Briefly dip one slide into into fresh pure xylene and *immediately* place 1–2 drops of EcoMount on the slide before the xylene dries.

   IMPORTANT! Use the EcoMount mounting medium only.

4. Carefully place 24 mm x 50 mm coverslip over the tissue section. Avoid trapping air bubbles.
5. Repeat steps 2 and 3 for each slide.
6. Air dry slides for at least **5 MIN**.
7. Proceed to **Chapter 5. Evaluate the Results** on page 25.

Recommended guidelines

We highly recommend following the guidelines for Cell Conditioning (Target Retrieval), and Pretreatment #3 (Protease) conditions for:

- Any new or previously untested FFPE tissue types.
- Samples prepared differently than the protocol in **Chapter 3. Prepare and Pretreat Samples** on page 14.

1. Stain six representative slides using the positive and negative control probes according to the following table:

Slide No.	Probe	Target Retrieval	Protease
1	Positive control	16 MIN	24 MIN
2	Negative control	16 MIN	24 MIN
3	Positive control	16 MIN	16 MIN
4	Negative control	16 MIN	16 MIN
5	Positive control	24 MIN	16 MIN
6	Negative control	24 MIN	16 MIN

2. Evaluate staining and tissue morphology as in **Chapter 5. Evaluate the Results** and determine which pretreatment condition yielded the highest positive control signal and lowest negative control signal. Using PPIB, positive control signal should have a staining score of 3 or higher, and the negative control signal should be 0.
3. Use the optimized pretreatment conditions to run the assay with the target probe.
4. If none of the conditions are satisfactory, contact technical support at **support.acd@bio-techne.com**.

5 Chapter 5. Evaluate the Results

Examine tissue sections under a standard bright field microscope at 20–40X magnification:

- Assess tissue and cell morphology.
- Assess positive control signal strength. Positive control signal should be visible as punctate dots within the cell cytoplasm at 20–40X magnification.
- Assess negative control background. One dot to every 10 cells displaying background staining per 40X microscope field is acceptable.
- Evaluate target probe signal using the scoring guidelines in the next section.

Scoring guidelines

The RNAscope® VS Duplex Assay enables a semi-quantitative scoring guideline utilizing the estimated number of punctate dots present within each cell boundary.

An example of how to develop such a guideline for semi-quantitative assessment of RNAscope® staining intensity is presented below for a gene with expression level varying between 1 to > 10 copies per cell.

Note: If your gene expression level is higher or lower than this range, you may need to scale the criteria accordingly.

Categorize staining into five grades: **0**, **1+**, **2+**, **3+**, and **4+** according to the following table:

Staining Score	Microscope Objective Scoring*
0	No staining, or less than 1 dot/10 cells (40X magnification)
1	1–3 dots/cell (visible at 20–40X magnification)
2	4–9 dots/cell. No or very few dot clusters (visible at 20–40X magnification)
3	10-15 dots/cell. Less than 10% positive cells have dot clusters (visible at 20X magnification)
4	>10 dots/cell. More than 10% positive cells have dot clusters (visible at 20X magnification)

* Discount cells with artificially high nuclear background staining.

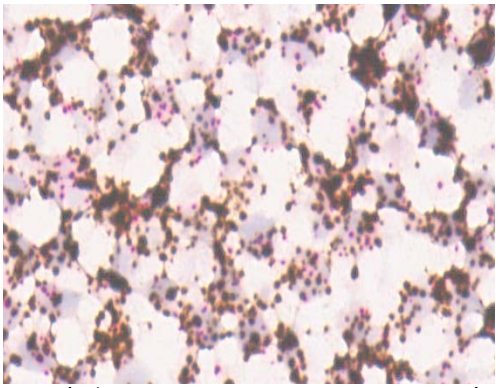
Troubleshooting

For troubleshooting information, please contact your local Field Application Scientist or technical support at **support.acd@bio-techne.com**.

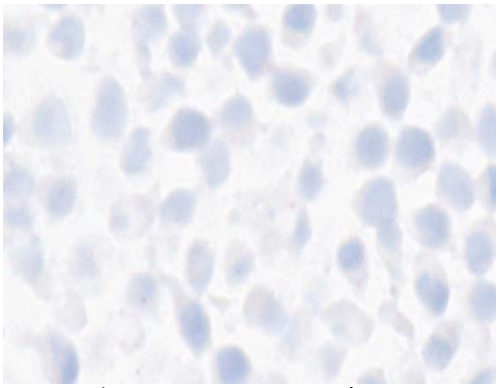
Tissue example

If the assay is successful, the staining should look like the following images:

Figure 2. RNAscope® VS Universal Assay results in HeLa cells



Hs-TBP (Red) / Hs-POLR2A (Brown) (Positive Control)



DapB / DapB (Negative Control)

A Appendix A. Semi-automated RNAscope® VS Duplex Assay

Most sample types can be fully automated on the Discovery ULTRA. Manual pretreatment may give a better result in some cases. Use the semi-automated procedure for tissues that do not have a satisfactory result when using the fully automated procedure. See **Chapter 4. Automated RNAscope® VS Duplex Assay** on page 16.

Workflow

Kit contents and storage

RNAscope Reagents

For Offline Boiling: RNAscope® Target Retrieval Reagents				
☑	Cat. No.	Reagent	Quantity	Storage
	322000	RNAscope® Target Retrieval Reagents	70 mL x 4 bottles	Room Temp (15–30°C)

* Not provided with the kit and needs to be purchased separately.

IMPORTANT! Do not substitute the reagent components of the RNAscope® VS Duplex Reagent Kit with those of other RNAscope® Reagent Kits, even those having the same name. The Target Retrieval solution in the RNAscope® VS Universal Sample Prep Kit CANNOT be used for offline boiling. Please separately purchase the RNAscope® Target Retrieval Reagents (Cat. No. 322000) to boil samples off the instrument.

Prepare the materials

Materials can be prepared ahead of time or while baking the slides, unless otherwise stated. See **Bake the slides** on page 32.

Materials required

Materials provided by Advanced Cell Diagnostics	Materials Provided by Ventana™ Medical Systems	Other Materials and Equipment
• RNAscope® 2.5 VS Target Probe • RNAscope® 2.5 VS Duplex Positive Control Probe • RNAscope® 2.5 VS Duplex Negative Control Probe • RNAscope® Target Retrieval Reagents • RNAscope® VS Protease • RNAscope® VS Duplex AMP 1 • RNAscope® VS Duplex AMP 2 • RNAscope® VS Duplex AMP 3 • RNAscope® VS Duplex AMP 4 • RNAscope® VS Duplex AMP 5 • RNAscope® VS Duplex AMP 6 • RNAscope® VS Duplex AMP 7 • RNAscope® VS Duplex AMP 8 • RNAscope® VS Duplex AMP 9 • RNAscope® VS Duplex AMP Wash • RNAscope® VS Hematoxylin • RNAscope® VS Bluing Reagent	• DISCOVERY™ ULTRA — automated slide stainer • DISCOVERY Wash 10x • ULTRA LCS (Predilute) • SSC Buffer 10X • DISCOVERY CC1 • Reaction Buffer 10X • Probe dispensers • mRNA Sample Prep Kit • mRNA Duplex Amp Kit • mRNA Red Detection Kit • mRNA DAB Detection Kit • mRNA Teal Detection Kit • User fillable dispensers • mRNA Link	• Distilled water • Glass beaker (1 or 2 L) • Hot plate • Dawn detergent or similar detergent • Fume hood • xylene • 100% ethanol (EtOH) • Tissue-Tek® Staining Dish (3) • Tissue-Tek® Clearing Agent Dish, xylene-resistant (3) • Tissue-Tek® Vertical 24 Slide Rack • EcoMount • Cover Glass, 24 mm x 50 mm

Prepare the instrument

If the instrument has not been used for ≥1 week, follow the guidelines for instrument maintenance in the *Ventana™ System User Manual*.

Dilute bulk reagents

Please prepare the bulk fluids as per manufacturer's instructions.

Register new reagents

Reagent dispensers come with appropriate barcode labels and registration buttons for dispensing RNAscope® VS Reagents. Refer to the *Ventana™ DISCOVERY ULTRA System User Manual* for details.
To register reagents:

1. Log all ACD reagents and probes into the software as "log user-fillable reagents" or "'log user-fillable probes".
2. Use the wand that comes with the instrument to register *new* reagent kits.

Prepare instrument reagents

Refer to the table on page 9 to determine the proper dispenser for each reagent.

1. For RNAscope® VS Duplex AMP 1 to AMP 9 and Amp Wash, transfer the entire volume of each component into the correspondingly labeled dispenser.
2. Transfer the remaining RNAscope® VS Duplex Reagents (VS Target Probe, VS Positive Control Probe, VS Negative Control Probe, VS Protease, VS Hematoxylin, and VS Bluing Reagent) to the correspondingly labeled dispensers.
3. Follow the dispenser product insert instructions to properly prime and handle the dispensers.
4. For the duplex target probe:
   a. Determine the volume of duplex probe needed
   b. In a clean container, add the appropriate volume of the 16X C2 probe stock to the desired volume of Ready-to-Use C1 probe. See the table below for some suggestions of volumes:

RTU C1 Volume	C2 Probe Stock Volume
1 mL	60 µL
2 mL	120 µL
3 mL	180 µL
4 mL	240 µL
5 mL	300 µL

   c. Invert the solution minimally 5 times to mix the probes.
   d. Transfer the solution to a clean Ventana open Probe dispenser.
   e. Properly prime the dispenser and place it on the instrument.
5. Store tightly-capped dispensers at **4°C** when not in use.
6. Check solution levels: DISCOVERY Wash, 2X SSC, Reaction Buffer, ULTRA LCS (Predilute), and CC1. Refill if they are less than half full. To run a full tray of 30 slides, fully fill the 2X SSC and ReactionBuffer containers.

IMPORTANT! Do not use expired reagents.

7. Empty the waste carboy if needed.

Prepare deparaffinization reagents

- In a fume hood, fill two clearing agent dishes with ~200 mL fresh xylene.
- In a fume hood, fill two staining dishes with ~200 mL fresh 100% EtOH.

Note: Ensure all containers remain covered when not in use.

Prepare 1X Target Retrieval

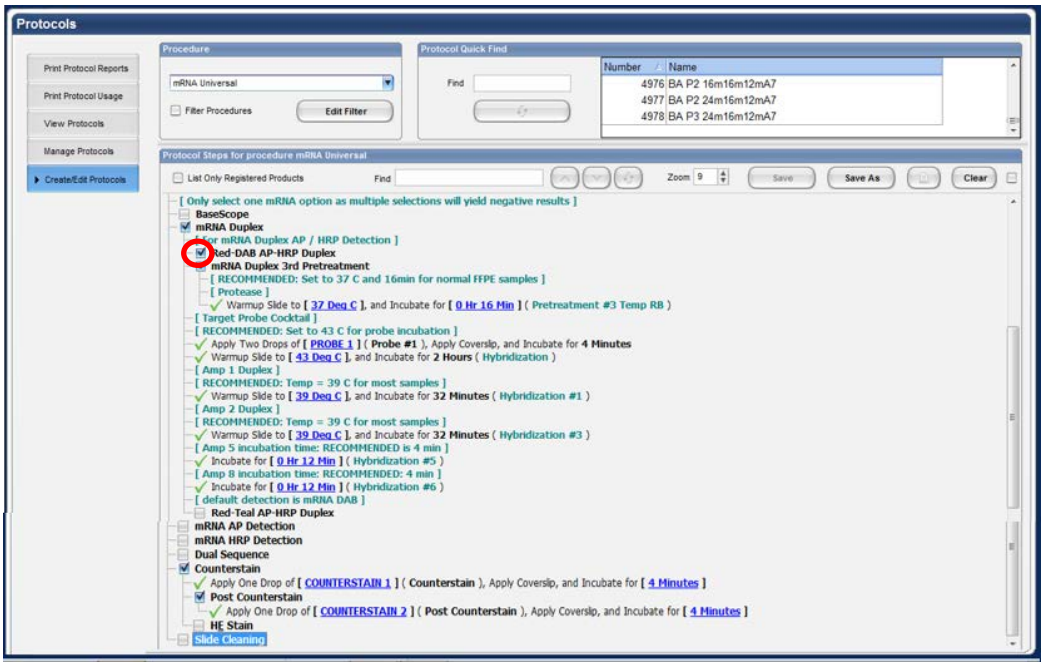
Prepare 1X Target Retrieval while FFPE slides are baking at 60°C, or the following day if you choose the optional stopping point on page 32. 1X Target Retrieval is used in manual cell conditioning (CC).

1. Prepare 700 mL of fresh 1X Target Retrieval by adding 630 mL distilled water to 1 bottle (70 mL) 10X 1X Target Retrieval solution in the beaker.
2. Mix well and cover the beaker with foil.

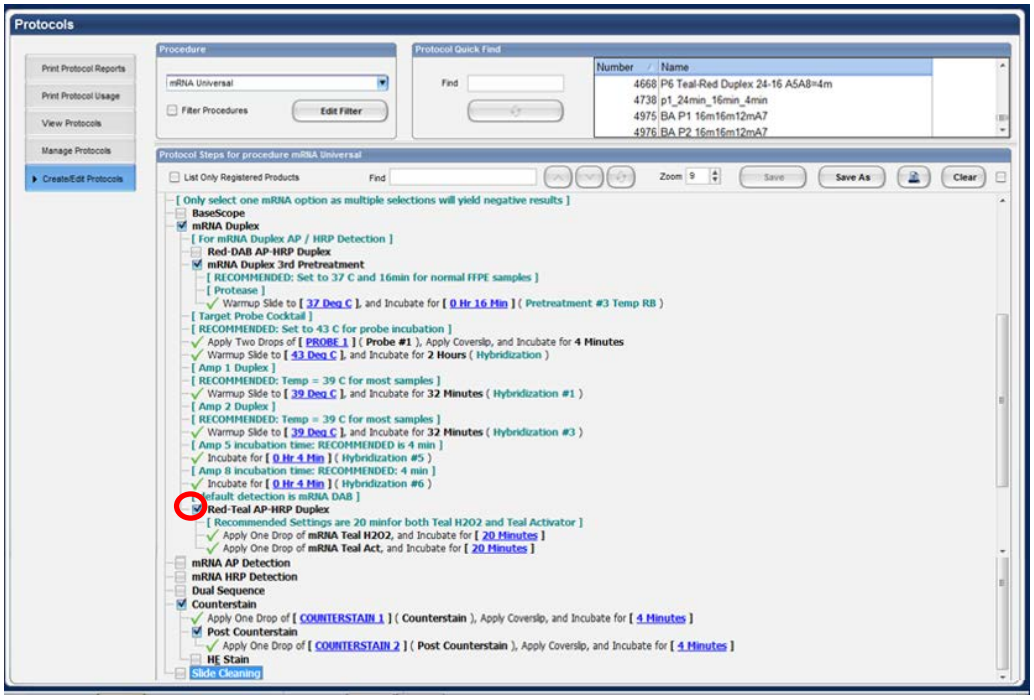
IMPORTANT! Do not use RNAscope® VS Universal Target Retrieval v2 for offline boiling.

Create an instrument protocol

1. Open the VSS software and click on the **Protocol** button.
2. Click on **Create/Edit Protocols**, go to the Procedure drop down menu and select **mRNA Universal**.
3. Main protocol steps appear as shown:
    a. For **Red**/**Brown**: After selecting the main protocol steps, drop down menus become available. Note: Make sure to select the Red-DAB AP-HRP Duplex box under the default detection is DAB line. Select the appropriate protocol steps by clicking on the associated check boxes as shown:



b. For **Red**/**Teal**: After selecting the main protocol steps, drop down menus become available. Note: Make sure to select the Red-Teal AP-HRP Duplex box under the *default detection is DAB* line. The suggested times for Teal incubation are *20 min mRNA Teal H_2O_2* and *20 min mRNA Teal Act*. Select the appropriate protocol steps by clicking on the associated check boxes as shown:



IMPORTANT! Do not select Baking, Deparaffinization, or Cell Conditioning.

4. After selecting the main protocol steps, drop down menus become available. Select the appropriate protocol steps by clicking on the associated check boxes as shown above.
5. Select the appropriate assay conditions from the drop-down menus according to the following table:

Suggested Temperatures/Times	
VS Protease	Protease: 37°C
Suggested probe temperatures	Single Probes 43°C
	Pooled Probes 50°C
Suggested AMP 1 and Amp 2 temperatures	39°C
AMP 5 incubation time*	4 MIN
AMP 8 incubation time*	4 MIN

* Staining intensity can be modified by adjusting Amp 5 (Red) and AMP 8 (Brown) incubation times.

6. Click **Save As**, then select a protocol number from the drop-down menu and choose a protocol name for each probe. Click **Save**.
7. Click **Close** to go back to the main screen.
8. Assign a probe number from the list to each probe of interest. For each probe selected, assign a protocol.

Print the labels

1. Select the **Print Label** icon from the bottom of the home page screen.
2. Select your preferred template or create a new template. To create a new template, refer to the *Ventana™ System User Manual* for details.
3. Select the protocol you created for the RNAscope® VS Duplex Assay.
4. Click on **Protocol** to add and print the label.

Manually pretreat the samples

Materials required

Materials Provided by the Target Retrieval Reagents (Cat. No. 322000)	Other Materials and Equipment
• RNAscope® Target Retrieval Reagents	• Drying oven • FFPE slides • Tissue-Tek® Vertical 24 Slide Rack • Distilled water • Fume hood • Xylene • 100% ethanol (EtOH) • Tissue-Tek® Clearing Agent Dish (2) • Tissue-Tek® Staining Dish (2) • Glass beaker (1 or 2 L) • Hot plate

Bake the slides

1. Bake slides in a dry oven for **30-60 MIN** at **60°C**.

OPTIONAL STOPPING POINT Use immediately or store at **RT** with desiccants for ≤1 week. Prolonged storage may degrade sample RNA.

IMPORTANT! If you continue, prepare the materials for the following protocols while the slides are baking: **Deparaffinize FFPE sections**, **Pretreat the slides**, and **Run the RNAscope® VS Duplex Assay**.

Deparaffinize FFPE sections

IMPORTANT! If you have not done so already, create a protocol for your instrument and print slide labels during this procedure. See page 30.

1. Place slides in a Tissue-Tek® Slide Rack and submerge in the first xylene-containing clearing agent dish in the fume hood.
2. Incubate the slides in xylene for **5 MIN** at **RT**. Agitate the slides by occasionally lifting the slide rack up and down in the clearing agent dish.
3. Remove the slide rack from the first xylene-containing dish and *immediately* place in the second xylene-containing clearing agent dish in the fume hood.
4. Repeat Step 2.

5. Remove the slide rack from the second xylene-containing dish and *immediately* place in the staining dish containing 100% EtOH.
6. Incubate the slides in 100% EtOH for **1 MIN** at **RT** with agitation.
7. Repeat Step 6 with fresh 100% EtOH.
8. Remove the slides from the rack, and place on absorbent paper with the section face-up. Air dry for **5 MIN** at **RT**.
9. While slides are drying, place printed labels on the slides.

IMPORTANT! Labels must be in place prior to the next section.

10. Insert the slides into a Tissue-Tek® Slide Rack and proceed to condition the slides.

Pretreat the slides

Begin heating 1X Target Retrieval Buffer while FFPE slides are baking at 60°C or during the previous section.

IMPORTANT! Do not boil 1X Target Retrieval more than **30 MIN** before use.

1. Heat 1X Target Retrieval Buffer to **98–104°C**:
   a. Place the beaker containing 1X Target Retrieval Buffer on the hot plate. Cover the beaker with foil and turn the hot plate on high for **10–15 MIN**.
   b. Once 1X Target Retrieval Buffer reaches a slow boil (**98–104°C**), turn the hot plate to a lower setting to maintain the correct temperature. Check the temperature with a thermometer.
2. With a pair of forceps *very slowly* submerge the slide rack containing the slides into the boiling 1X Target Retrieval Buffer solution. Cover the beaker with foil and boil the slides for the amount of time specified in the following table:

Tissue Type	Target Retrieval Time
Brain and spinal cord	15 MIN
Breast cancer	15 MIN
Cell lines	10 MIN
Colon	15 MIN
GI tract	15 MIN
Head and neck cancer	15 MIN
Heart	15 MIN
Kidney	15 MIN
Liver	30 MIN
Lung	15 MIN
Lymphoma	10 MIN
Placenta	15 MIN
Prostate	15 MIN
Skin	15 MIN
Stomach	15 MIN
Thymus	10 MIN
Tonsil	10 MIN

Tissue Type	Target Retrieval Time
Xenograft derived from cell lines	7 MIN
Xenograft derived from primary tumor	15 MIN

3. Use the forceps to *immediately* transfer the hot slide rack from the 1X Target Retrieval Buffer to a staining dish containing distilled water. Do not let the slides cool in Target Retrieval.
4. Wash slides 3–5 times by moving the Tissue-Tek® Slide Rack up and down in the distilled water.
5. Repeat Step 4 with fresh distilled water.
6. Proceed directly to **Load the reagents** on page 34.

Run the RNAscope® VS Duplex Assay

Materials required

- Prepared slides
- Prepared instrument reagents
- Prepared detergent
- Distilled water
- Prepared dehydrating materials
- Tissue-Tek® Vertical 24 Slide Rack
- EcoMount
- Cover Glass, 24 mm x 50 mm

Load the reagents

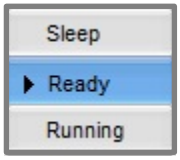
1. Remove the nozzle caps from the filled dispensers and place each cap on the post located on the back of the dispenser.
2. If needed, remove any air bubbles at the nozzle tips by pushing down on the nozzle until the liquid reaches the tip of the nozzle or forms a small meniscus at the tip of the nozzle

IMPORTANT! Do not dispense any drops as this could compromise your drop inventory.

3. Load dispensers onto the reagent racks.
4. Remove the yellow locking ring from the dispensers in the prefilled mRNA RED Detection and mRNA DAB Detection or mRNA Teal Detection Kits. Refer to the instructions provided by Ventana™ Medical Systems.
5. Load the reagent racks onto the reagent carousel.

Start the run

1. Click the **Ready** button.

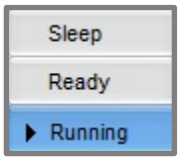


2. Eject slide drawers.

3. Load each slide onto a heater pad with the label facing upward and inward. Ensure that the slides sit securely on the pads.

IMPORTANT! Prior to loading the slides, ensure heater pads are completely dry. Wipe off any solution using laboratory tissue paper.

4. Close slide drawers.
5. Click the **Running** button. Semi- automated assay will finish in **~12 HRS**.



IMPORTANT! Before leaving the instrument unattended, ensure all reagents and slides are successfully registered and the instrument is running.

Prepare detergent

1. Prepare 200 mL of diluted detergent by adding 1 to 2 drops detergent to 200 mL distilled water in a container with a cap.
2. Mix well by inverting the container 4–5 times.
3. Add diluted detergent to a Tissue-Tek® Staining Dish.
   Note: Store diluted detergent at RT.

Prepare dehydrating reagents

IMPORTANT! Do not use deparaffinization solutions for dehydration.

- In a fume hood, fill a clearing agent dish with ~200 mL fresh xylene.
  Note: Ensure all containers remain covered when not in use.

Complete the run

1. After the run is complete, place nozzle caps back on the dispensers.
2. Store reagent racks at **4°C** until next use.

IMPORTANT! Store the Dewax dispenser at room temperature.

Wash the slides

1. Submerge a Tissue-Tek® Slide Rack into the Tissue-Tek® Staining Dish containing 200 mL diluted detergent.
2. Open the instrument slide drawer and unload slides.
3. Decant solution on the slides into the slide drawer, then *immediately* load slides into the Tissue-Tek® Slide Rack submerged in detergent.
4. Rinse oil off the slides by moving the slide rack up and down in the dish 10 times.
5. Replace the detergent with distilled water and rinse slides by moving the slide rack up and down a minimum of 10 times.
6. Repeat Step 5 three to five times.

Mount the samples

1. Remove the slide rack from the staining dish and dry slides in a **60°C** dry oven for **30 MIN**.

IMPORTANT! The RED substrate is alcohol sensitive. Do not dehydrate the slides in alcohol.

2. Cool the slides for **5 MIN** at **RT**.
3. Briefly dip one slide into into fresh pure xylene and *immediately* place 1–2 drops of EcoMount on the slide before the xylene dries.

IMPORTANT! Use the EcoMount mounting medium only.

4. Carefully place 24 mm x 50 mm coverslip over the tissue section. Avoid trapping air bubbles.
5. Repeat steps 2 and 3 for each slide.
6. Air dry slides for at least **5 MIN**.
7. Proceed to **Chapter 5. Evaluate the Results**.

Recommended guidelines

We highly recommend following the guidelines for Cell Conditioning (Target Retrieval) and Protease conditions for:

- Any new or previously untested FFPE tissue types.
- Samples prepared differently than the protocol in **Chapter 3. Prepare and Pretreat Samples** on page 14.

1. Stain six representative slides using the positive and negative control probes according to the following table:

Slide No.	Probe	Target Retrieval	Protease
1	Positive control	10 MIN	24 MIN
2	Negative control	10 MIN	24 MIN
3	Positive control	10 MIN	16 MIN
4	Negative control	10 MIN	16 MIN
5	Positive control	15 MIN	16 MIN
6	Negative control	15 MIN	16 MIN

2. Evaluate staining and tissue morphology as in **Chapter 5. Evaluate the Results**, and determine which pretreatment condition yielded the highest positive control signal and lowest negative control signal. Using PPIB, positive control signal should have a staining score of 3 or higher, and the negative control signal should be 0.
3. Use the optimized pretreatment conditions to run the assay with the target probe.
4. If none of the conditions are satisfactory, contact technical support at **support.acd@bio-techne.com**.

B Appendix B. Safety

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see **https://acdbio.com/technical-support/user-manuals**.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT**! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **https://www.cdc.gov/biosafety/**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10051&p_table=STANDARDS**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

- Additional information about biohazard guidelines is available at: **https://www.cdc.gov/biosafety/**

In the EU:

- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at:
  http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/
- Information about the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) can be found at: **https://echa.europa.eu/regulations/reach**

Documentation and Support

Obtaining support

For the latest services and support information, go to: **https://acdbio.com/technical-support/support-overview.**

At the website, you can:

- Access telephone and fax numbers to contact Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Search for user documents, SDSs, application notes, citations, training videos, and other product support documents.
- Find out information about customer training events.

Contact information

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Newark, CA 94560
Toll Free: 1-877-576-3636
Direct: 1-510-576-8800
Fax: 1-510-576-8801
Information: **info.acd@bio-techne.com**
Orders: **orders.acd@bio-techne.com**
Support Email: **support.acd@bio-techne.com**

Limited product warranty

Advanced Cell Diagnostics, Inc. and/or its affiliate(s) warrant their products as set forth in the ACD General Terms and Conditions of Sale found on the ACD website. If you have any questions, please contact Advanced Cell Diagnostics at **https://acdbio.com/about/contact**.

Headquarters
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For support, email support.acd@bio-techne.com.
www.acdbio.com