USER MANUAL

BaseScope™ VS Assay

For Ventana™ DISCOVERY™ ULTRA System

RED

Document Number UM 323700

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1 Chapter 1. Product Information

Before using this product, read and understand the information in **Appendix B. Safety** of 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 BaseScope VS Assay:

- **Chapter 4. Automated BaseScope VS Assay** starting on page 15.
- **Appendix A. Semi-automated BaseScope VS Assay** starting on page 26.

Product description

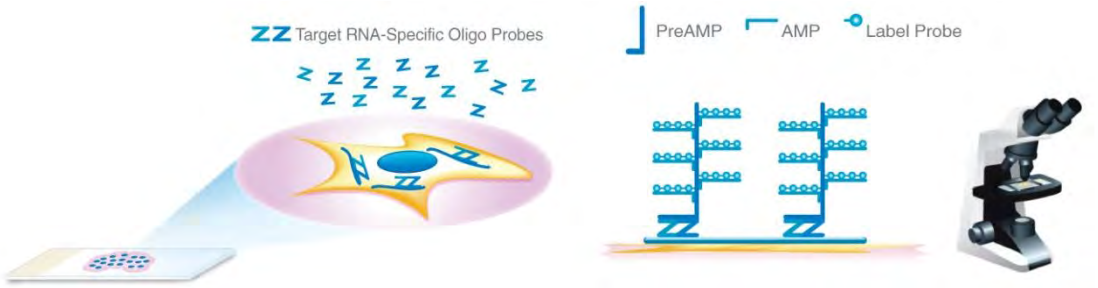
Background

The BaseScope Assay uses a novel and proprietary method of *in situ* hybridization (ISH) to visualize single RNA molecules for splice variants and short targets in samples mounted on slides. BaseScope Assays do not require the RNA-free environment used for traditional ISH. The assays are based on ACD's patented signal amplification and background suppression technology. Compared with the RNAscope® 2.5 Assay, the BaseScope Assay incorporates an additional signal amplification step, which makes it possible to detect RNA splicing variants, point mutations, small insertions or deletions, and short RNA targets (50–300 nucleotides).

Overview

Figure 1 on page 7 illustrates the BaseScope VS Assay procedure. You can complete the procedure on the Ventana DISCOVERY ULTRA System in ~12–13 hours. Starting with properly prepared samples, sections are first pretreated, and then RNA-specific probes are hybridized to target RNA. Amplify the signal using multiple steps, followed by hybridization to alkaline phosphatase (AP)-labeled probes and detection using a red chromogenic substrate. Each single RNA transcript appears as a distinct dot of chromogen precipitate visible when using a common bright-field microscope at 40X magnification.

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. The BaseScope Assay enhances the signal with additional amplification steps. Add **Fast Red** substrate to detect target RNA.	Visualize target RNA with a standard bright field microscope.

Kit contents and storage

The BaseScope VS Assay requires the BaseScope VS Probes and the BaseScope VS Detection Reagent Kit. Probes and Reagent Kits are available separately.

IMPORTANT! BaseScope VS Probes must be used with the BaseScope VS Detection Reagent Kit. RNAscope VS probes are incompatible with the BaseScope Detection Reagent Kit.

BaseScope VS Probes

The BaseScope 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 to stain ~30 standard slides. The probes have a shelf life of two years from the manufacturing date when stored as indicated in the following tables:

Target Probes					
☑	Reagent	Cat. No.	Content	Quantity	Storage
	BaseScope VS Target Probe *([species] – [gene]*	Various	Probe targeting specific RNA	7 mL x 1 bottle	2–8°C
Control Probes					
☑	Reagent	Cat. No.	Content	Quantity	Storage
	BaseScope VS Positive Control Probe-Human (Hs)-PPIB-3ZZ	701039	Probe targeting common housekeeping gene	7 mL x 1 bottle	2–8°C
	BaseScope VS Positive Control Probe-Mouse (Mm)-Ppib-3ZZ	701079	Probe targeting common housekeeping gene	7 mL x 1 bottle	2–8°C
	BaseScope VS Positive Control Probe-Human (Hs)-PPIB-1ZZ	701049	Probe targeting common housekeeping gene	7 mL x 1 bottle	2–8°C
	BaseScope VS Positive Control Probe-Mouse (Mm)- Ppib-1ZZ	701089	Probe targeting common housekeeping gene	7 mL x 1 bottle	2–8°C
	BaseScope VS Negative Control Probe- DapB-3ZZ	701019	Probe targeting bacterial gene dapB	7 mL x 1 bottle	2–8°C
	BaseScope VS Negative Control Probe- DapB-1ZZ	701029	Probe targeting bacterial gene dapB	7 mL x 1 bottle	2–8°C

IMPORTANT! When running the BaseScope VS assay, make sure that your control probes contain the same number of ZZ pairs as your target probe. Consult support at **support.acd@bio-techne.com.**

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 BaseScope VS Positive Control Probes and the BaseScope VS Negative Control Probes. The slides have a shelf life of nine months from the manufacturing date when stored at 2–8°C with desiccants.

BaseScope VS Reagents

BaseScope VS Reagent kits provide enough reagents to stain ~60 standard slides. You will receive two kits when you order the BaseScope VS Reagent Kit (Cat. No. 323700). BaseScope VS Reagents include:

- BaseScope VS Detection Reagents (Cat. No. 323710)
- RNAscope Universal VS Sample Preparation Reagents v2 (PN 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 manufacturing date when stored as indicated in the following tables:

BaseScope VS Detection Reagents (Cat. No. 323710)				
☑	Reagent	Cat. No.	Quantity	Storage
	BaseScope VS AMP 1	323711	14 mL x 1 bottle	2–8°C
	BaseScope VS AMP 2	323712	14 mL x 1 bottle	2–8°C
	BaseScope VS AMP 3	323713	14 mL x 1 bottle	2–8°C
	BaseScope VS AMP 4	323714	14 mL x 1 bottle	2–8°C
	BaseScope VS AMP 5	323715	14 mL x 1 bottle	2–8°C
	BaseScope VS AMP 6	323716	14 mL x 1 bottle	2–8°C
	BaseScope VS AMP 7	323717	14 mL x 1 bottle	2–8°C
	BaseScope VS AMP 8	323718	14 mL x 1 bottle	2–8°C
	RNAscope VS Protease	322218	14 mL x 1 bottle	2–8°C
RNAscope VS Universal Sample Prep Reagents v2 (Cat. No. 323740)				
☑	Reagent	Cat. No.	Quantity	Storage
	RNAscope VS Universal Target Retrieval v2	323741	14 mL x 2 bottles	Room Temp (15–30°C)
	RNAscope VS Universal Dewax	323742	14 mL x 1 bottle	Room Temp (15–30°C)
RNAscope VS Accessory Kit (Cat. No. 320630)				
☑	Reagent	Cat. No.	Quantity	Storage
	RNAscope VS Hematoxylin	320631	7 mL x 1 bottle	2–8°C
	RNAscope VS Bluing Reagent	320632	7 mL x 1 bottle	2–8°C

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

IMPORTANT! BaseScope VS and RNAscope VS Universal assays share some of the same reagents including RNAscope VS Protease, RNAscope VS Target Retrieval v2, RNAscope VS Universal Dewax, RNAscope VS Hematoxylin, and RNAscope VS Bluing Reagent. Only these reagents can be interchanged among kits. Do not interchange other reagents.

Required materials from Roche Diagnostics

The BaseScope VS 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 BaseScope VS Probes. Use up to 20 probes at one 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 dispenser — 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 RED Probe Amplification Kit (Cat. No. 760-236; Ordering Code 7095341001)		
☑	**Component**	**Storage**
	ACD RED AMP 1 dispenser — fill dispenser with BaseScope VS AMP 1	Room Temp (15–30°C)
	ACD RED AMP 2 dispenser — fill dispenser with BaseScope VS AMP 2	Room Temp (15–30°C)
	ACD RED AMP 3 dispenser — fill dispenser with BaseScope VS AMP 3	Room Temp (15–30°C)
	ACD RED AMP 4 dispenser — fill dispenser with BaseScope VS AMP 4	Room Temp (15–30°C)
	ACD RED AMP 5 dispenser — fill dispenser with BaseScope VS AMP 5	Room Temp (15–30°C)
	ACD RED AMP 6 dispenser — fill dispenser with BaseScope VS AMP 6	Room Temp (15–30°C)
	ACD RED AMP 7 dispenser — fill dispenser with BaseScope VS AMP 7	Room Temp (15–30°C)
Ancillary Dispensers (Cat. No.771-758, Ordering Code 05271916001)		
☑	**Component**	**Storage**
	250 Test Option #8 — fill dispenser with BaseScope VS AMP 8	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 05271738001)		
☑	**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 VS Bluing Reagent	Room Temp (15–30°C)

Equipment and buffers

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

IMPORTANT! To run the BaseScope VS assay successfully, use DISCOVERY Wash (950-510) or EasyPrep 10X (950-102). Place 2X SSC (950-110) in the SSC bulk container. 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 Dishes	American Master Tech Scientific/MLS	LWT4457EA
	Tissue-Tek Clearing Agent Dishes, xylene resistant	American Master Tech Scientific/MLS	LWT4456EA
	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 BaseScope VS 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 BaseScope Positive and Negative Control Probes.

Important procedural guidelines

- Start with properly fixed and prepared sections. Refer to
- **Chapter 3. Prepare and Pretreat** Samples **and .Recommended guidelines** on page 22
- 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 36 in this document for more information.

3 Chapter 3. Prepare and Pretreat Samples

Formalin-fixed, paraffin-embedded (FFPE) sample preparation is described in the following protocol.

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

For samples treated differently from the following protocol, you may need to optimize pretreatment conditions. Refer to **Recommended guidelines** on page 22, 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 Universal Assay.

Dehydrate, embed, and cut the sample

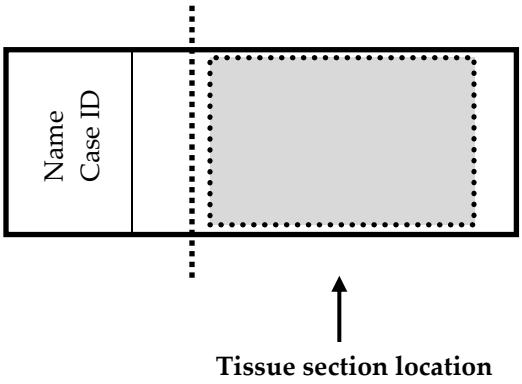
IMPORTANT! Use fresh reagents.

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), we recommend storing at 2–8°C with desiccation.

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 BaseScope within one week.

OPTIONAL STOPPING POINT Store sections with desiccants, either at room temperature for up to three months, or at –20°C–4°C for up to six months.

4 Chapter 4. Automated BaseScope VS Assay

IMPORTANT! We strongly recommend you run the RNAscope VS Control Slides (Cat. No. 310045 or 310023) using the BaseScope VS Positive and Negative Control Probes along with your samples in every run.

Appendix A. Semi-automated BaseScope VS Assay on page 26 describes an offline boiling procedure for use with Cat. No.322000 (RNAscope Target Retrieval Reagents).

Workflow

Prepare the materials

⬇

Run the BaseScope VS Assay ~13 HRS

⬇

Wash and mount slides

Prepare the materials

Materials required

Materials Provided by Advanced Cell Diagnostics	Materials Provided by Ventana Medical Systems	Other Materials and Equipment
• BaseScope VS Target Probe • BaseScope VS Positive Control Probe • BaseScope VS Negative Control Probe • RNAscope VS Universal Dewax • RNAscope VS Target Retrieval • RNAscope VS Protease • BaseScope VS AMP 1 • BaseScope VS AMP 2 • BaseScope VS AMP 3 • BaseScope VS AMP 4 • BaseScope VS AMP 5 • BaseScope VS AMP 6 • BaseScope VS AMP 7 • BaseScope VS AMP 8 • RNAscope VS Hematoxylin • RNAscope VS Bluing Reagent	• DISCOVERY ULTRA — automated slide stainer • DISCOVERY Wash Buffer 10X • ULTRA LCS (Predilute) • SSC Buffer 10X • DISCOVERY CC1 • Reaction Buffer (10X) • Probe dispensers • mRNA Sample Prep Kit • mRNA Red Probe Amplification Kit • mRNA Red Detection Kit • User fillable dispensers • Option 8 dispenser	• Distilled water • Dawn detergent or similar detergent • Fume hood • Xylene • Tissue-Tek Staining Dish • Tissue-Tek Clearing Agent Dish, xylene-resistant • 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 more than a 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 BaseScope 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 tables on pages 9–10 to determine the proper dispenser for each reagent.

1. For BaseScope VS reagents AMP1 to AMP 7, transfer the entire volume of each component into the correspondingly labeled mRNA Red Probe Amplification Kit dispenser.
2. For BaseScope VS AMP 8, transfer the entire volume into the Option 8 dispenser.

3. Transfer the BaseScope VS Target Probe, BaseScope VS Positive Control Probe, BaseScope VS Negative Control Probe, RNAscope VS Universal Dewax, RNAscope VS Protease, both bottles of RNAscope VS Target Retrieval v2, RNAscope VS Hematoxylin, and RNAscope 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.

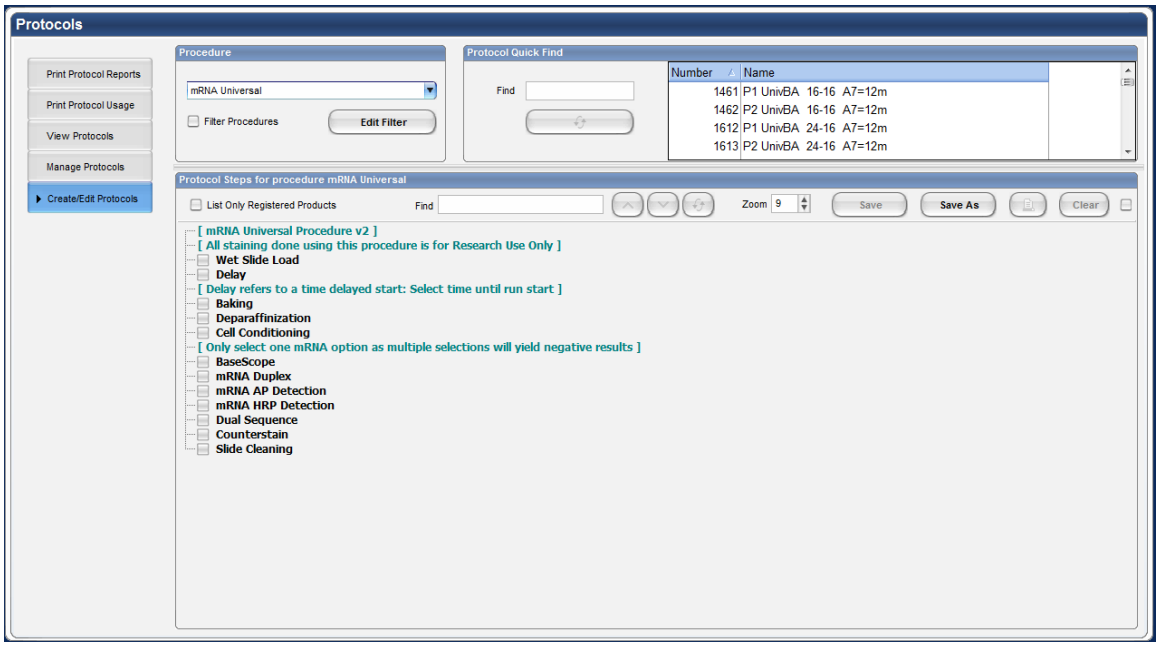
4. Follow the dispenser product insert instructions to properly prime and handle the dispensers.
5. Store tightly-capped dispensers (except the mRNA Dewax dispenser) at **4°C** when not in use. Store the tightly-capped mRNA Dewax dispenser at **15–30°C**.

IMPORTANT! Do not use expired reagents.

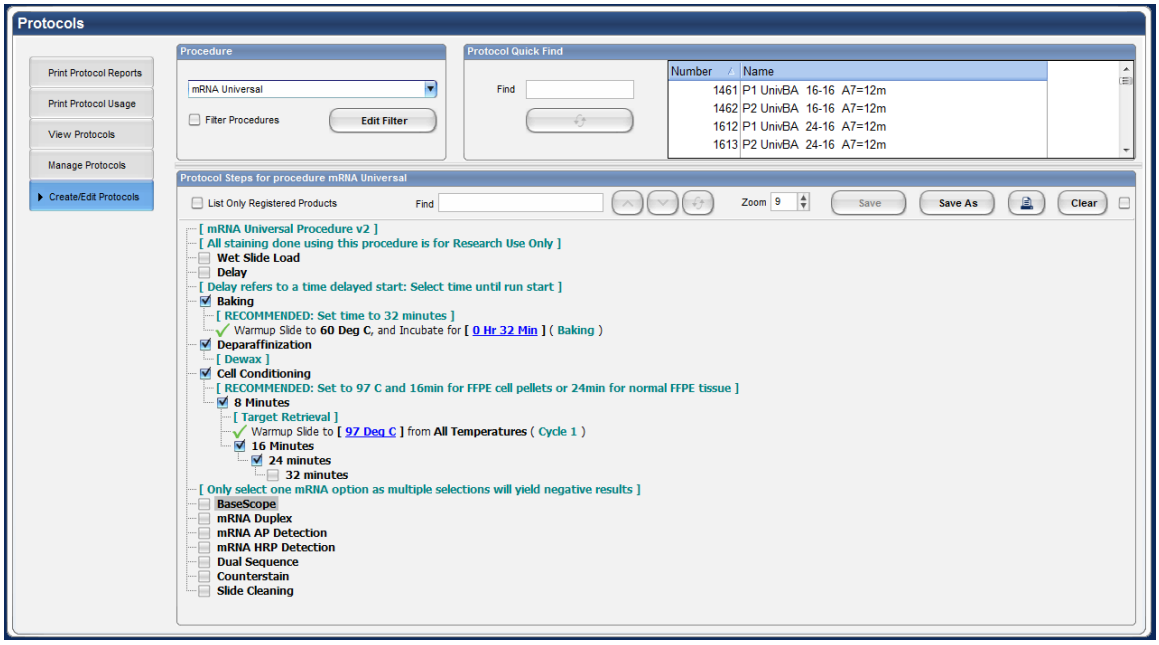
6. Empty the waste bottle if needed.

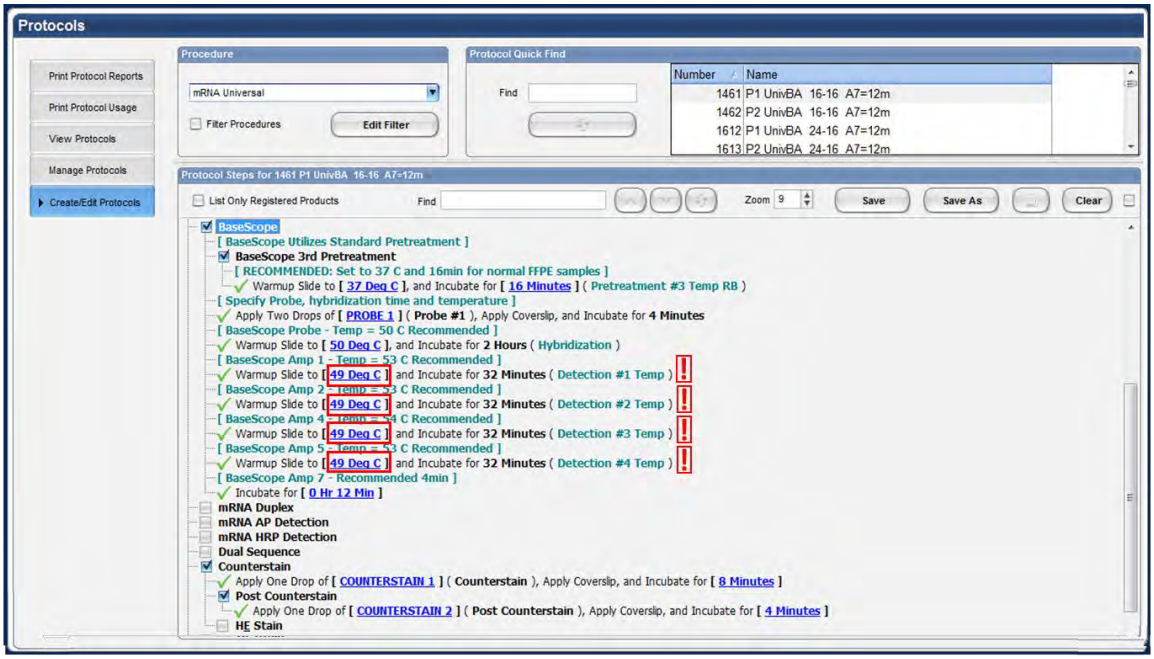
Create an instrument protocol

1. Open the VS 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. Select the appropriate assay conditions from the drop down menus according to the following tables:

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

Standard Temperatures/Times	
VS Protease	37°C, 16 MIN
Standard probe temperatures	50°C
Standard BaseScope AMP 1 temperature	49°C†
Standard BaseScope AMP 2 temperature	49°C†
Standard BaseScope AMP 4 temperature	49°C†
Standard BaseScope AMP 5 temperature	39°C†‡
Standard BaseScope AMP 7 incubation time*	12 MIN

*Calibrate the staining intensity for your instrument using the BaseScope AMP 7 incubation time. We suggest titrating signal intensity using incubation times of 12 MIN, 24 MIN, and 36 MIN. If the instrument settings have been previously optimized for the mRNA Universal software, you can use the same time setting that you used for RNAscope AMP 5.

† **IMPORTANT!** These are the latest recommendations from ACD and can differ from the recomemndations given by the VS software.

‡ If a hazy nuclear background is observed please increase the Amp 5 incubation temperature to 41°C.

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 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 BaseScope VS 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 BaseScope VS 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 Kit.** Refer to the instructions provided by Ventana Medical Systems.
5. Load the reagent racks onto the reagent carousel.

Start the run

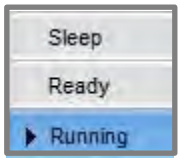
1. Click the **Ready** button.

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 ~**12-13 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 four to five 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 dispenser, 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 mRNA 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 24.

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 13.

IMPORTANT! VS pretreatment reagents and conditions are shared across BaseScope and RNAscope assays. If pretreatment conditions have been previously established for a given tissue with the RNAscope Universal assay, the same pretreatment conditions may be applied to the BaseScope VS assay.

1. Stain four 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	16 MIN
2	Negative control	16 MIN	16 MIN
3	Positive control	24 MIN	16 MIN
4	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 the lowest negative control signal. Using 1zz PPIB, the positive control signal should have a staining score of 1 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 first pretreatment conditions are satisfactory, proceed to the following table for further optimization:

Slide No.	Probe	Target Retrieval	Protease
1	Positive control	24 MIN	32 MIN/50°C
2	Negative control	24 MIN	32 MIN/50°C
3	Positive control	48 MIN	16 MIN/37°C
4	Negative control	48 MIN	16 MIN/37°C
3	Positive control	48 MIN	32 MIN/50°C
4	Negative control	48 MIN	32 MIN/50°C

5. If no satisfactory pretreatment conditions can be found, please 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 BaseScope 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 BaseScope 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/20 cells (40X magnification)
1	1 dot/cell (visible at 20–40X magnification)
2	2–3 dots/cell. No or very few dot clusters (visible at 20–40X magnification)
3	4–10 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.

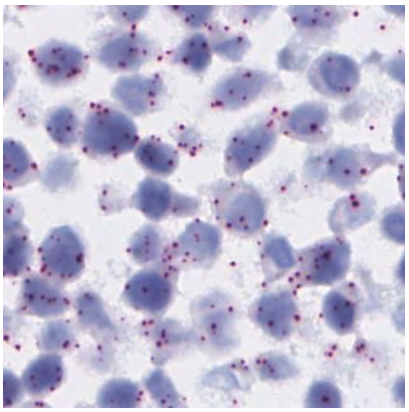
Troubleshooting

For troubleshooting information, please contact technical support at support.acd@bio-techne.com.

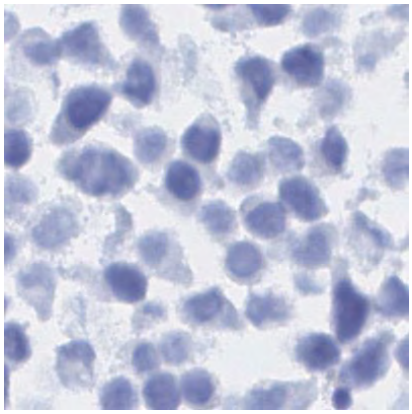
Staining example

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

Figure 2. BaseScope VS Assay results in HeLa cells



1zz Hs-POLR2A (Positive Control)



1zz DapB (Negative Control)

Appendix A. Semi-automated BaseScope VS 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 BaseScope VS Assay** on page 15.

Workflow

Prepare the materials

⬇

Bake the slides

⬇

Deparaffinize FFPE sections

⬇

Pretreat the slides

⬇

Run the BaseScope VS Assay ~10 HRS

⬇

Wash and mount slides

Kit contents and storage

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 BaseScope VS Reagent Kit with those of other BaseScope or RNAscope Reagent Kits, even those having the same name. The Target Retrieval solution in the BaseScope VS Reagent 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 31.

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 Positive Control Probe • RNAscope 2.5 VS Negative Control Probe • RNAscope Target Retrieval Reagents • RNAscope VS Protease • BaseScope VS AMP 1 • BaseScope VS AMP 2 • BaseScope VS AMP 3 • BaseScope VS AMP 4 • BaseScope VS AMP 5 • BaseScope VS AMP 6 • BaseScope VS AMP 7 • BaseScope VS AMP 8 • RNAscope VS Hematoxylin • RNAscope VS Bluing Reagent	• DISCOVERY ULTRA — automated slide stainer • DISCOVERY Wash • ULTRA LCS (Predilute) • SSC Buffer 10X • Reaction Buffer • DISCOVERY CC1 • Probe dispensers • mRNA Sample Prep Kit • mRNA RED Probe Amplification Kit • mRNA Red Detection Kit • Option 8 dispenser • User fillable dispensers	• Distilled water • Glass beaker (1 or 2 L) • Hot plate • Dawn detergent or similar detergent • Fume hood • Xylene • 100% ethanol (EtOH) • Tissue-Tek Staining Dishes • Tissue-Tek Clearing Agent Dishes, xylene-resistant • 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 more than a 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 BaseScope 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 tables on pages 9–10 to determine the proper dispenser for each reagent.

1. For BaseScope VS AMP 1 to AMP 7, transfer the entire volume of each component into the correspondingly labeled mRNA Red Probe Amplification kit dispenser.
2. For BaseScope VS AMP 8, transfer the entire volume into the Option 8 dispenser.
3. Transfer the BaseScope VS Target Probe, BaseScope VS Positive Control Probe, BaseScope VS Negative Control Probe, VS Protease, VS Hematoxylin, and VS Bluing Reagent to the correspondingly labeled dispensers.
4. Follow the dispenser product insert instructions to properly prime and handle the dispensers.
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 Reaction Buffer 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 31. 1X Target Retrieval is used in manual cell conditioning (CC).

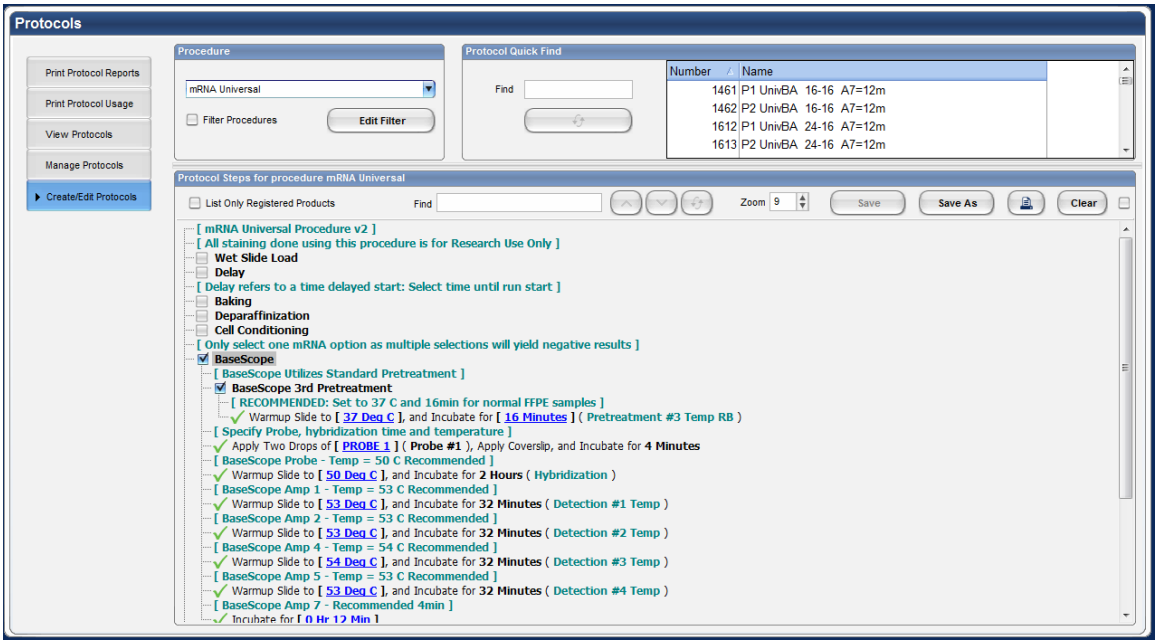
1. Prepare 700 mL of fresh 1X Target Retrieval by adding 630 mL distilled water to 1 bottle (70 mL) 10X 1X Target Retrieval solution in the beaker.
2. Mix well and cover the beaker with foil.

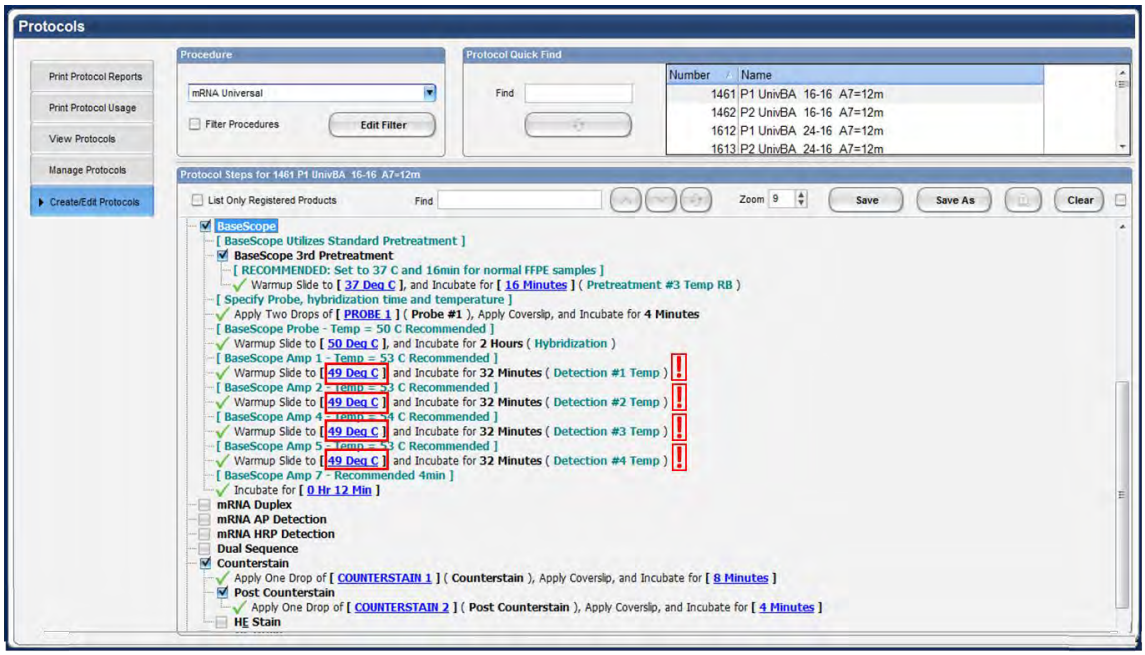
IMPORTANT! Do not use RNAscope VS Universal Target Retrieval v2 for offline boiling.

Create an instrument protocol

1. Open the VS 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:





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 tables:

Standard Temperatures/Times	
VS Protease	37°C, 16 MIN
Standard probe temperatures	50°C
Standard BaseScope AMP 1	49°C†
Standard BaseScope AMP 2	49°C†
Standard BaseScope AMP 4	49°C†
Standard BaseScope AMP 5	49°C†‡
Standard BaseScope AMP 7 incubation time*	4 MIN

*Calibrate the staining intensity for your instrument using the BaseScope AMP 7 incubation time. We suggest titrating signal intensity using incubation times of 4 MIN, 12 MIN, and 24 MIN. If the instrument settings have been previously optimized for the mRNA Universal software, you can use the same time setting that you used for RNAscope AMP 5.

† **IMPORTANT!** These are the latest recommendations from ACD and can differ from the recomemndations given by the VS software.

‡ If a hazy nuclear background is observed please increase the Amp 5 incubation temperature to 53°C.

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 BaseScope VS 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 • Tissue-Tek Staining Dish • 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 BaseScope VS 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 pages 28–30.

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.
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
Xenograft derived from cell lines	7 MIN
Xenograft derived from primary tumor	15 MIN

3. 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. Remove the slides from the rack and place on absorbent paper with the section face-up. Air dry for **5 MIN** at **RT**.
7. While slides are drying, place printed labels on the slides.

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

8. Proceed directly to **Load the reagents**.

Run the BaseScope VS 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 Kit.** Refer to the instructions provided by Ventana Medical Systems.
5. Load the reagent racks onto the reagent carousel.

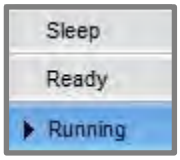
Start the run

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 **~10 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.

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 13.

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 1zz PPIB, positive control signal should have a staining score of 1 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 SDSs

Safety Data Sheets (SDSs) are available at: **https://acdbio.com/technical-support/user-manuals**. For the SDSs of chemicals not distributed by Advanced Cell Diagnostics, contact the chemical manufacturer.

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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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**. I**f you have any questions, please contact Advanced Cell Diagnostics at **https://acdbio.com/about/contact**.

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7707 Gateway Blvd Suite 200, Newark, CA 94545 Phone 1-510-576-8800 Toll Free 1-877-576-3636
For support, email **support.acd@bio-techne.com**
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