USER MANUAL

Formalin-Fixed Paraffin-Embedded (FFPE) Sample Preparation and Pretreatment

For the RNAscope® 2.5 Assay

PART 1

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Citing RNAscope® 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.

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Contents

1 Chapter 1. Product Information

Before using this product, read and understand the information in **Appendix C. Safety** on page 20.

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

About this guide

This user manual provides guidelines and protocols for the proper preparation and pretreatment of formalin-fixed, paraffin-embedded (FFPE) tissues mounted on slides. The slides can then be assayed using an RNAscope® 2.5 Reagent Kit.

RNAscope® 2.5 Reagent Kits come with a separate RNAscope® 2.5 Assay User Manual. Do not perform an RNAscope® 2.5 Assay without the correct user manual.

Visit **https:// acdbio.com/technical-support/user-manuals** to download RNAscope® 2.5 Assay User Manuals.

Product description

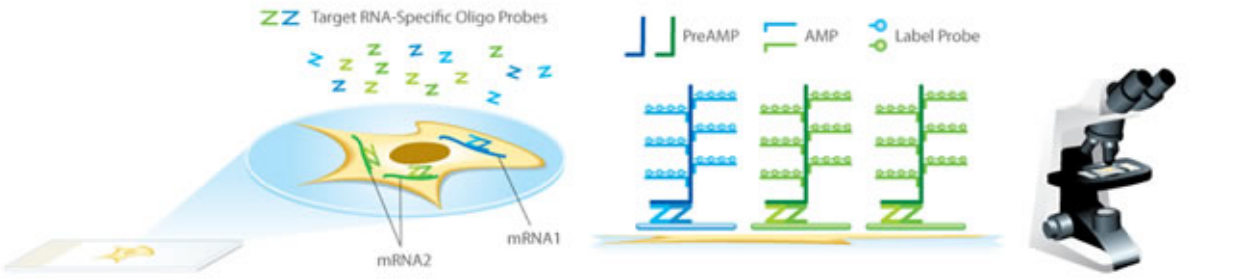
Background

The RNAscope® 2.5 Assays use a novel and proprietary method of *in situ* hybridization (ISH) to visualize single RNA molecules per cell in samples mounted on slides. The assays are based on ACD's patented signal amplification and background suppression technology. Proprietary RNA-specific probes are hybridized to target RNA, and are then bound to a cascade of signal amplification molecules culminating in signal detection. Single-plex, 2-plex, multiplex, and automated assays are all available. **Figure 1** on page 6 illustrates the RNAscope® 2.5 Assay procedure. The procedure can be completed in 6–10 hours depending on the assay, or conveniently divided over two days. Most RNAscope® 2.5 Assay reagents are available in convenient Ready-To-Use (RTU) dropper bottles and provide a simple, nearly pipette-free workflow. Observe results using standard bright field or fluorescent microscopy.

Sample types

To perform the RNAscope® 2.5 Assays, you must start with properly prepared and pretreated samples. Multiple sample types are now compatible with RNAscope® 2.5 Assays and include: formalin-fixed, paraffin-embedded (FFPE) tissues; fresh, frozen tissues; fixed, frozen tissues; tissue microarray (TMA); and cell samples. Visit **www.acdbio.com** and contact **support@acdbio.com** for more information.

Figure 1. Procedure overview



1: Tissue section	2: Hybridize to target RNA	3: Amplify signal	4: Image
Start with properly prepared tissue sections and pretreat to allow access to target RNA.	Hybridize two sets of gene-specific probe pairs to target mRNA.	Use two independent signal amplification systems to detect both target RNAs. Probes are hybridized to a cascade of signal amplification molecules, culminating in binding of HRP- or AP-labeled probes. Add two chromogenic substrates to detect RNAs.	Visualize target RNA using a standard bright-field microscope.

Kit contents and storage

RNAscope® 2.5 Assay Reagents

RNAscope® Assays require RNAscope® Probes and an RNAscope® 2.5 Reagent Kit. If you are performing an automated assay using the Ventana® DISCOVERY XT or ULTRA Systems, you must use RNAscope® VS Probes and the RNAscope® 2.5 VS Reagent Kit. Probes and Reagent Kits are available separately. RNAscope® HD Assays offer the choice of two Detection Reagents, Brown (DAB) and Red (Fast Red), which enable RNA molecules to be visualized as brown or red chromogenic dots, respectively. Visit **https://acdbio.com/products** to find a gene-specific Target Probe or appropriate Control Probes.

RNAscope® 2.5 Reagent Kit

Each RNAscope® 2.5 Reagent Kit provides enough reagents to stain ~20 tissue sections approximately 20 mm x 20 mm large. Larger tissue sections will result in fewer tests.

Each kit includes RNAscope® Target Retrieval Reagents (Cat No. 322000) and separately, RNAscope® Hydrogen Peroxide and RNAscope® Protease Plus (Cat. No. 322330). Pretreatment instructions are provided in this guide. For information on the other reagents and directions for use, refer to an RNAscope® 2.5 Assay User Manual. The reagents have a shelf life of nine months from the date of bulk manufacturing when stored as indicated in the following table:

☑	Reagent	Cat. No.	Quantity	Storage
	RNAscope® Hydrogen Peroxide	322335	3 mL x 2 bottles	2–8°C
	RNAscope® Protease Plus	322331	4.5 mL x 1 bottle	2–8°C
	RNAscope® Target Retrieval Reagents	322000	70 mL x 4 bottles	15–30°C

IMPORTANT! RNAscope® 2.5 Reagent Kits share the same Wash Buffer, but have unique pretreatment reagents and Detection Kits. Do not interchange the reagent components of the Detection Kits, even those having the same name.

Required materials and equipment

The following materials and equipment are needed to perform the RNAscope® 2.5 Assay.

HybEZ™ Hybridization System

IMPORTANT! The RNAscope® 2.5 Assay has been verified using this system only.

Use the HybEZ™ Hybridization System to perform RNAscope® Assay hybridization and incubation steps. These steps require humid conditions to prevent sections from drying out.

For instructions on how to use the HybEZ™ Hybridization System, refer to the *HybEZ™ Hybridization System User Manual* available at **https://acdbio.com/documents/support-documents** and view the training video at **https://acdbio.com/technical-support/learn-more**. The system contains the following components:

☑	Component	Quantity	Cat. No.
	HybEZ™ Oven (110 or 220 VAC) or HybEZ™ II Oven (110 or 220V)	1 oven	310010 or 310013 (HybEZ™) 321710 or 321720 (HybEZ™ II)
	HybEZ™ Humidity Control Tray (with lid)	1 tray	310012
	ACD EZ-Batch™ Slide Rack (20 slide capacity)	1 rack	310017
	HybEZ™ Humidifying Paper	2 sheets	—

Note: To order HybEZ™ Humidifying Paper Pack, 15 sheets, use Cat. No. 310015.

User-supplied materials

IMPORTANT! Do not substitute other materials for the ImmEdge™ Hydrophobic Barrier Pen and the SuperFrost® Plus Slides listed in the following table.

☑	Description	Supplier	Cat. No.
	ImmEdge™ Hydrophobic Barrier Pen (required)	Vector Laboratory	H-4000
	SuperFrost® Plus Slides (required)	Fisher Scientific	12-550-15
	Xylene	Fisher Scientific/MLS*	X3P-1GAL
	100% alcohol (EtOH)	American Master Tech Scientific/MLS	ALREACS
	10% neutral-buffered formalin (NBF)	MLS	—
	Paraffin wax	MLS	—
	Microtome	MLS	—
	Drying oven, capable of holding temperature at 60 +/– 1°C	MLS	—
	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 Desish, xylene resistant	American Master Tech Scientific/MLS	LWT4456EA
	Water bath or incubator, capable of holding temperature at 40 +/– 1°C	MLS	—

☑	Description	Supplier	Cat. No.
	Distilled water	MLS	—
	Tubes (various sizes)	MLS	—
	Fume hood	MLS	—
	Paper towel or absorbent paper	MLS	—
	Glass beaker 1 or 2 L (Optional)†	MLS	—
	Hot plate (Optional)†	Fisher Scientific/MLS	11-300-49SHP
	Aluminum foil (Optional)†	MLS	—
	Forceps, large (Optional)†	MLS	—
	Digital thermometer	MLS	—
	Oster® Steamer Model 5712, Black and Decker Steamer HS3000, or the Braun Multiquick FS 20 Steamer		

* Major Laboratory Supplier in North America. For other regions, please check Catalog Numbers with your local lab supplier.
† Required for the alternate target retrieval method in **Appendix B** on page 19.

2 Chapter 2. Before You Begin

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

- View the video demonstrations available at **https://acdbio.com/technical-support/learn-more**.
- Run the assay on FFPE RNAscope® 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.
- Use only samples mounted on SuperFrost Plus® Slides (Fisher Scientific; Cat. No. 12-550-15).
- Follow the recommended pretreatment conditions for your sample. Refer to **Appendix A. Tissue Pretreatment Recommendation** on page 17.
- Always run positive and negative control probes on your sample to assess sample RNA quality and optimal assay workflow.
- 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 (except after Target Retrieval ethanol wash).
- Use good laboratory practices and follow all necessary safety procedures. Refer to **Appendix B. Safety** on page 20 for more information.

3 Chapter 3. Prepare and Pretreat Samples

The following protocols describe formalin-fixed, paraffin-embedded (FFPE) sample preparation and pretreatment. For other sample types and preparation methods, contact **support@acdbio.com** for the latest protocols and guidelines.

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

For suboptimally treated samples, you may need to optimize pretreatment conditions. Refer **Appendix A. Tissue Pretreatment Recommendation** on page 17in addition to **https://acdbio.com/technical-support/solutions**.

Prepare FFPE sections

Materials required

- 10% neutral buffered formalin (NBF)
- 1X PBS
- Paraffin wax
- 100% alcohol (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® 2.5 Assay.

Dehydrate, embed, and cut the sample

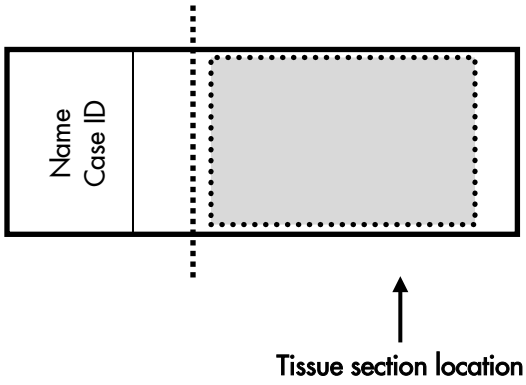
IMPORTANT! Use fresh reagents.

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 room temperature 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**.

OPTIONAL STOPPING POINT. Use sectioned tissue within 3 months. Store sections with dessicants at room temperature.

Prepare FFPE slides for the RNAscope® 2.5 Assay

IMPORTANT! Do not use the HybEZ™ Oven.

Workflow

Bake slides~1 HR

↓

Optional Stopping Point

↓

Deparaffinize FFPE sections ~20 MIN

↓

Optional Stopping Point for single plex assays

IMPORTANT! We do not recommend stopping the protocol for duplex assays until after hybridizing your sample with probe. Refer to PART 2, RNAscope 2.5 Duplex Detection 322500-USM.

Materials required

- Drying oven
- Prepared FFPE slides
- Tissue-Tek® Vertical 24 Slide Rack
- Distilled water
- Fume hood
- Xylene
- 100% alcohol (EtOH)
- Tissue-Tek® Clearing Agent Dish (2)
- Tissue-Tek® Staining Dish (2)

Bake slides

1. Bake slides in a dry oven for **1 HR** at **60°C**.

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

2. If you continue, prepare the materials for the following protocols while the slides are baking: **Deparaffinize FFPE sections** in the next section, **Pretreat samples** on page 12, and RNAscope® 2.5 assay materials.

Note: If you choose the optional stopping point following **Create a barrier** on page 14, prepare the reagents for **Apply RNAscope® Protease Plus** on page 16 and RNAscope® 2.5 assay materials the next day.

Deparaffinize FFPE sections

Reagents may be prepared ahead of time. Ensure all containers remain covered.

1. In a fume hood:
   - Fill two Tissue-Tek® Clearing Agent dishes with ~200 mL fresh xylene.
   - Fill two Tissue-Tek® Staining dishes with ~200 mL fresh 100% alcohol.
2. Place slides in a Tissue-Tek® Slide Rack and submerge in the first xylene-containing dish in the fume hood.
3. Incubate the slides in xylene for **5 MIN** at **RT**. Agitate the slides by occasionally lifting the slide rack up and down in the dish.
4. Remove the slide rack from the first xylene-containing dish and *immediately* place in the second xylene-containing dish in the fume hood.
5. Incubate the slides in xylene for **5 MIN** at **RT** with agitation.
6. Remove the slide rack from the second xylene-containing dish and *immediately* place in the dish containing 100% alcohol.
7. Incubate the slides in 100% alcohol for **1 MIN** at **RT** with agitation.
8. Remove the slide rack from the first alcohol-containing dish, and *immediately* place in the second alcohol-containing dish.
9. Incubate the slides in 100% alcohol for **1 MIN** at **RT** with agitation.
10. Remove the slides from the rack, and place on absorbent paper with the section face-up. Air dry slides for **5 MIN** at **RT** (or until completely dry).

Pretreat samples

IMPORTANT! Before you begin, make sure you know the pretreatment conditions specific to your sample type from **Appendix A. Tissue Pretreatment Recommendation** on page 17.

Workflow

Prepare 1X RNAscope® Target Retrieval Reagents ~10–20 MIN

↓

Apply RNAscope® Hydrogen Peroxide~10 MIN

↓

Perform target retrieval ~10–30 MIN

↓

Create a barrier ~15 MIN

↓

Optional Stopping Point for single plex assays

IMPORTANT! We do not recommend stopping the protocol for duplex assays until after hybridizing your sample with probe. Refer to PART 2, RNAscope 2.5 Duplex Detection 322500-USM.

↓

Apply RNAscope® Protease Plus ~30–60 MIN

↓

PROCEED **IMMEDIATELY** TO RNASCOPE® 2.5 ASSAY

Materials required

Materials provided by the RNAscope® 2.5 Reagent Kit	Other Materials and Equipment
• RNAscope® Hydrogen Peroxide • RNAscope® Target Retrieval Reagents • RNAscope® Protease Plus	• Prepared slides • Distilled water • HybEZ™ Humidifying System/ACD EZ-Batch™ Slide Rack • Paper towel or absorbent paper • Steamer • Digital thermometer • Tissue Tek® Slide Rack • Tissue Tek® Staining Dishes • ImmEdge™ Hydrophobic Barrier Pen

Equilibrate equipment

1. Turn on HybEZ™ Oven and set temperature to **40°C**.
2. Place a Humidifying Paper in the Humidity Control Tray and wet completely with distilled water.
3. Insert covered tray into oven and close the oven door. Warm the tray for **30 MIN** at **40°C** before use. Keep the tray in the oven when not in use.

Prepare 1X RNAscope® Target Retrieval Reagents

1) Prepare 200 mL of fresh RNAscope® 1X Target Retrieval Reagents by adding 180 mL distilled water to 20 mL 10X Target Retrieval Reagents. Mix well.

Apply RNAscope® Hydrogen Peroxide

1. Lay deparaffinized slides on the bench and add ~5–8 drops of RNAscope® Hydrogen Peroxide to cover the entire section.
2. Incubate slides for **10 MIN** at **RT**.
3. Remove RNAscope® Hydrogen Peroxide solution from one slide at a time by tapping and/or flicking the slide on absorbent paper. Immediately insert the slide into a Tissue-Tek® Slide Rack submerged in a Tissue-Tek® Staining Dish filled with distilled water.
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.

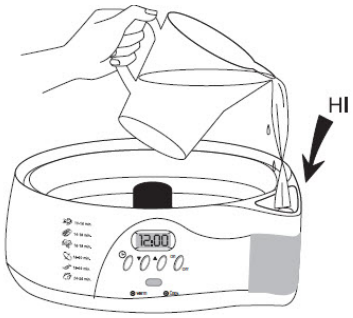
Perform target retrieval

We highly recommend using an Oster® Steamer for target retrieval. For an alternate method, see **Appendix B. Manual Target Retrieval** on page 19.

Note: You may also steam with the Braun Multiquick FS 20 Steamer or the Black and Decker Steamer HS3000. To use the Braun Multiquick FS 20 Steamer, fill the water to the maximum level before starting and do not refill water during the steaming process.

1. Fill the water reservoir with cold tap water to the "HI" marking line.

IMPORTANT! Do not overfill.

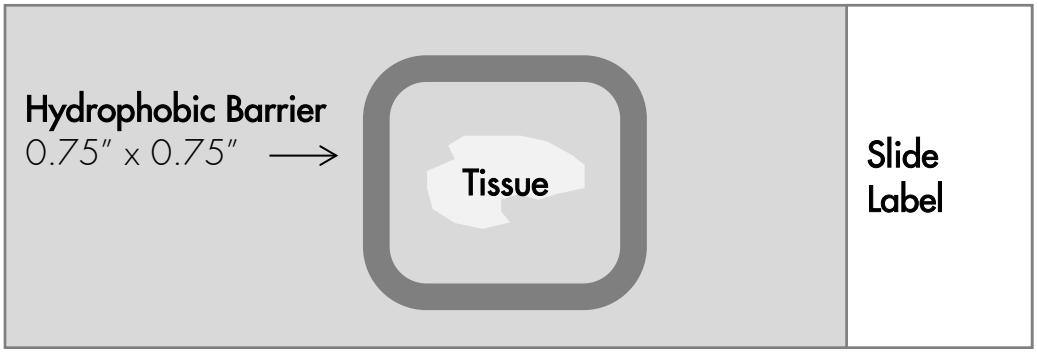


2. Place a clear Steaming Bowl onto the base.
3. Place two slide holders in the steam bowl. Fill one slide holder with 200 mL of RNAscope® 1X Target Retrieval Reagent. Fill the other slide holder with 200 mL of distilled H_20.
4. Turn on the Steamer. Set the steamer timer by turning the black knob clockwise. Set heating time to **95 MIN**.
5. Insert a digital thermometer through the holes of the lid and into the container containing RNAscope® 1X Target Retrieval Reagent. Allow temperature to rise to at least **99°C**.
6. Add slides to the container containing distilled H_20 for 10 seconds to acclimate slides.
7. Remove slides and move them to the container containing RNAscope® 1X Target Retrieval Reagent. Cover the Steamer with lid.
8. Start timer for **15 MIN** for mild and standard conditions, and **30 MIN** for extended pretreatment. For pretreatment times, consult **Appendix A. Tissue Pretreatment Recommendation** on page 17.
9. Remove slides from steamer and transfer to a separate rinse container with 200 mL of distilled water. Allow slides to rinse for **15 SEC**.
10. Transfer slides to 100% alcohol for **3 MIN**.
11. Dry slides in **60°C** incubator (or at room temperature).

Create a barrier

1. Use the following template to draw a barrier 2–4 times around each section with the Immedge™ hydrophobic barrier pen.

IMPORTANT! Do not let the barrier touch the tissue section. An Immedge™ hydrophobic barrier pen is highly recommended. An alternative type of pen may result in suboptimal results. Do not let the barrier touch the tissue section.



Note: We do not recommend drawing a smaller barrier and using less than the recommended volume amounts, even for smaller sections. Larger barriers will result in fewer tests per kit.

2. Let the barrier dry completely **~1 MIN** or **OVERNIGHT** at **RT**.

Note: If you need to reapply the hydrophobic barrier during the following procedures, dry the appropriate area of the slide with a kimwipe. Do not touch the tissue section.

OPTIONAL STOPPING POINT. Dry slides overnight for use the following day, or proceed directly to the next section.

IMPORTANT! We do not recommend stopping the protocol for duplex assays until after hybridizing your sample with probe. Refer to PART 2, RNAscope 2.5 Duplex Detection 322500-USM.

Apply RNAscope® Protease Plus

1. Place dried slides on the HybEZ™ Slide Rack, and add ~5 drops of RNAscope® Protease Plus to entirely cover each section.
2. Remove the HybEZ™ Humidity Control Tray from the HybEZ™ Oven and place the HybEZ™ Slide Rack in the tray. Close the lid, seal, and insert tray back into the oven. Incubate at **40°C** for the amount of time specified by the table in **Appendix A. Tissue Pretreatment Recommendation** on page 17.

Note: If needed, prepare RNAscope® 2.5 assay materials during this step.

3. Remove the HybEZ™ Humidity Control Tray from the oven. Remove the HybEZ™ Slide Rack from the tray. Place tray back into the oven.
4. Take each slide one at a time from the HybEZ™ Slide Rack and tap/and or flick to remove the excess liquid. Immediately place each slide in a Tissue-Tek® Slide Rack submerged in a Tissue-Tek® Staining Dish filled with distilled water.
5. Wash slides 3–5 times by moving the Tissue-Tek® Slide Rack up and down in the distilled water.

Proceed to the RNAscope® 2.5 Assay

While the slides are incubating, proceed *immediately* to an RNAscope® 2.5 Assay. Ensure you have the appropriate user manual. User manuals are available at **https:// acdbio.com/technical-support/user-manuals**.

Troubleshooting

For troubleshooting information, please contact technical support at **support@acdbio.com**.

Appendix A. Tissue Pretreatment Recommendation

Follow the recommended pretreatment conditions based on your tissue type for:

- Any new or previously untested FFPE tissue types.
- Samples prepared differently than the sample preparation protocol found in this user manual.

Tissue pretreatment recommendation

1. Stain representative samples using the positive and negative control probes.
2. Fix sample in fresh 10% NBF for **16–32 HRS** at **RT**.

Note: Perform tissue fixation step using the recommended amount of time. Over or under-fixation will result in significant signal loss when performing the RNAscope® Assay.

3. Depending on your tissue type, vary the amount of time for the Target Retrieval Reagents and/or Protease Plus. Refer to the following section.

Reagent	Mild	Standard	Extended
RNAscope® Target Retrieval Reagents	15 MIN	15 MIN	30 MIN
RNAscope® Protease Plus	15 MIN	30 MIN	30 MIN

Note: Some sample types, such as certain Xenografts and Cell Pellets, may require less time. For these tissue types, vary the RNAscope® Target Retrieval Reagents time to **8 MIN** and RNAscope® Protease Plus time to **15 MIN**. For the ACD Cell Pellet sample, we recommend a **10 MIN** treatment with Target Retrieval Reagents, and a **30 MIN** treatment with RNAscope® Protease Plus. If you have a tissue type not listed, contact support at **support@acdbio.com**.

Tissue-specific pretreatment conditions

For suboptimally treated samples, you may need to optimize pretreatment conditions. Refer this document and information provided at http://acdbio.com/technical-support/solutions.

If your sample fixation is successful in fresh 10% NBF (see Step 2 from the preceding protocol), then refer to the following table for tissue-specific pretreatment conditions. For information about species or tissue type not listed here, contact support at **support@acdbio.com**.

Species	Tissue Type	Pathology	Pretreatment Condition
Mouse/Rat	Intestine	Normal	Standard
	Intestine	Tumor	Standard
	Embryo	Normal	Standard
	Brain	Normal	Standard
	Spleen	Normal	Mild
	Eye/Retina	Normal	Standard
	Liver	Normal	Extended
	Kidney	Normal	Standard

Species	Tissue Type	Pathology	Pretreatment Condition
Human	Breast	Tumor	Standard
	Colon	Tumor	Standard
	Colon	Normal	Standard
	Lung	Tumor	Standard
	Lung	Normal	Standard
	Prostate	Tumor	Standard
	Prostate	Normal	Standard
	Lymph node	Tumor	Standard
	Lymph node	Normal	Standard
	Tonsil	Normal	Standard
	Pancreas	Normal	Standard
	Cervical	Cancer	Standard
	Cervical	Normal	Standard
	Cervical dysplasia	Abnormal	Standard
	Brain	Tumor	Standard
	Brain	Normal	Standard
	Head	Cancer	Standard
	Neck	Cancer	Standard
	Liver	Cancer	Standard
	Kidney	Normal	Standard
	Skin	Normal	Standard
	Melanoma	Tumor	Standard
	Nevus	Benign	Standard
	Placenta	Normal	Standard
	Skin (TMA*)	Normal	Standard
	Breast (TMA)	Normal	Standard
	Melanoma (TMA)	Normal	Standard
	Nevus (TMA)	Benign	Standard
	Stomach (TMA)	Normal	Standard
	Stomach (TMA)	Tumor	Standard
	Cell pellets, fixed with 10% NBF	—	Mild
	HeLa cells, fixed with 10% Formaldehyde/PBS/ACD Control	—	10 MIN Target Retrieval; 30 MIN Protease Plus

* Tissue Microarray

B Appendix B. Manual Target Retrieval

Materials required

Materials provided by the RNAscope® 2.5 Reagent Kit	Other Materials and Equipment
• RNAscope® 10X Target Retrieval Reagents	• Prepared slides • Distilled water • Glass beaker (1 or 2 L) • Paper towel or absorbent paper • Hot plate, isotemp brand • Aluminum foil • Thermometer • Forceps, large • Tissue Tek® Slide Rack • Tissue Tek® Staining Dish • ImmEdge™ Hydrophobic Barrier Pen

Prepare 1X RNAscope® Target Retrieval Reagents

IMPORTANT! Do not boil the 1X RNAscope® Target Retrieval Reagents more than **15 MIN** before use.

1. Prepare 700 mL of fresh RNAscope® 1X Target Retrieval Reagents by adding 630 mL distilled water to 1 bottle (70 mL) 10X Target Retrieval Reagents in the beaker. Mix well.
2. Place the beaker containing RNAscope® 1X Target Retrieval Reagents on the hot plate. Cover the beaker with foil and turn the hot plate on high for **10–15 MIN**.
3. Once 1X RNAscope® Target Retrieval Reagents reaches a mild boil (**98–102°C**), turn the hot plate to a lower setting to maintain the correct temperature. Check the temperature with a thermometer.

Apply RNAscope® Target Retrieval Reagents

1. With a pair of forceps *very slowly* submerge the slide rack containing the slides into the mildly boiling RNAscope® 1X Target Retrieval Reagents solution. Cover the beaker with foil and boil the slides for the amount of time specified by the table in **Appendix A. Tissue Pretreatment Recommendation** on page 17.
2. Use the forceps to *immediately* transfer the hot slide rack from the RNAscope® 1X Target Retrieval Reagents to the staining dish containing distilled water. Do not let the slides cool in the Target Retrieval Reagents solution.
3. Wash slides 3–5 times by moving the Tissue-Tek® Slide Rack up and down in the distilled water.
4. Wash slides in fresh 100% alcohol and allow the slides to dry completely at **RT**.
5. Draw hydrophobic barrier and continue with the assay.

C Appendix C. 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, **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

Advanced Cell Diagnostics, Inc.
7707 Gateway Blvd Suite 200
Newark, CA 94545
Toll Free: 1-877-576-3636
Direct: 1-510-576-8800
Fax: 1-510-576-8801
Information: **info@acdbio.com**
Orders: **orders@acdbio.com**
Support Email: **support@acdbio.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
7707 Gateway Blvd Suite 200, Newark, CA 94545 Phone 1-510-576-8800 Toll Free 1-877-576-3636
For support, email **support@acdbio.com**
www.acdbio.com