USER MANUAL

ADVANCED CELL DIAGNOSTICS, INC.

RNAscope® Sample Preparation and Pretreatment Guide for FFPE Tissue

PART 1

Catalog Number 320511

*Compatible with the following User Manuals: RNAscope® 2.0 HD Detection Kit – BROWN (**Catalog No.** 320497), RNAscope® 2.0 HD Detection Kit – RED (**Catalog No.** 320487), and RNAscope® 2-Plex Detection Kit – Chromogenic (**Catalog No.** 320494).*

For Molecular Biology Applications, not intended for diagnosis.

Trademarks

RNAscope® and HybEZ™ are trademarks of Advanced Cell Diagnostics, Inc. All other trademarks belong to their respective owners.

Citing RNAscope® 2.0 in Publications

When describing a procedure for publication using this product, please refer to it as the RNAscope® 2.0 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

Advanced Cell Diagnostics, Inc. reserves the right to change its products and services at any time to incorporate technological developments. This manual is subject to change without notice.

Although this manual has been prepared with every precaution to ensure accuracy, Advanced Cell Diagnostics, Inc. assumes no liability for any errors, omissions, or for any damages resulting from the use of this information.

Copyright

Contents

1 Chapter 1. Product Information

Before using this product, read and understand the information in **Appendix C. Safety** on page 23 in this document.

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® Detection Kit.

RNAscope® Reagent Kits come with a separate RNAscope® Assay User Manual. Do not perform an RNAscope® Assay without the correct user manual. Refer to the manuals available at www.acdbio.com/support/technical-doc for more details. For any questions, please contact support@acdbio.com.

Product description

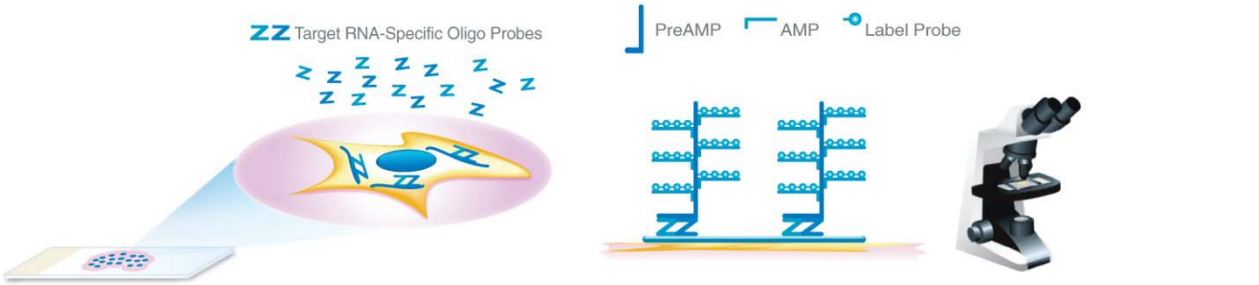
Background

The RNAscope® 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 (Figure 1). Single-plex, 2-plex, multiplex and automated assays are all available. The RNAscope® Assay procedure is illustrated in Figure 1 on page 6 and can be completed in 6–10 hours depending on the assay, or conveniently divided over two days. Most RNAscope® Assay reagents are available in convenient Ready-To-Use (RTU) dropper bottles and provide a simple, nearly pipette-free workflow. Results are observable using standard bright field or fluorescent microscopy.

Sample types

In order to perform the RNAscope® Assays, you must start with properly prepared and pretreated samples. Multiple sample types are now compatible with RNAscope® Assays and include: formalin-fixed paraffin-embedded (FFPE) tissues, fresh frozen tissues, fixed frozen tissues, tissue microarray (TMA), and cultured cells. 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 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 2.0 Assay enhances signal further with additional amplification steps. Add DAB or Fast Red substrate to detect target RNA.	Visualize target RNA using a standard bright field microscope.

RNAscope® Assay reagents

RNAscope® Assays require the RNAscope® Probes and an RNAscope® Detection 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® VS Reagent Kit. Probes and Reagent Kits are available separately.

Visit **www.acdbio.com/products/target-probes/search-product** to find a gene-specific probe from a searchable catalog of >27,000 predesigned Target Probes, or order a custom probe. Visit **www.acdbio.com/products/target-probes/controls-housekeeping** to find appropriate Control Probes.

RNAscope® Reagent Kit

Each RNAscope® Detection 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 contains three sub-kits including a Pretreatment Kit (Cat. no. 310020). Pretreatment Kit instructions are provided in this guide. For information on the other sub-kits and directions for use, refer to an RNAscope® Detection Kit User Manual. The reagents have a shelf life of six months from the shipment date when stored as indicated in the following table:

Pretreatment Kit (Cat. no. 310020)			
☑	Reagent	Quantity	Storage
	Pretreat 1 — Ready-To-Use (RTU) endogenous blocker	4 mL x 2 bottles	4°C
	10X Pretreat 2*	70 mL x 4 bottles	Room temperature (20–25°C)
	Pretreat 3 — RTU† protease	4.5 mL x 1 bottle	4°C

* Comes in a separate box.
† Dilute Pretreat 3 for some samples. See **Appendix A. Tissue Pretreatment Guidelines** on page 19.

Required materials and equipment

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

HybEZ™ Hybridization System

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

The HybEZ™ Hybridization System (110 VAC, Cat. no. 310010; 220 VAC, Cat. no. 310013) is designed for the hybridization and incubation steps in the RNAscope® Assays. Incubation steps in the RNAscope® Assay 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 **www.acdbio.com/support/technical-doc** and view the training video at **www.acdbio.com/support/online-training-videos**. The system contains the following components:

☑	Component	Quantity	Cat. no.
	HybEZ™ Oven (110 or 220 VAC)	1 oven	310010 or 310013
	HybEZ™ Humidity Control Tray (with lid)	1 tray	310012
	HybEZ™ Slide Rack (20 slide capacity)	1 rack	310014
	HybEZ™ Humidifying Paper	2 sheets	—
	HybEZ™ Humidifying Paper Pack	15 sheets	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
	100% ethanol	American Master Tech Scientific/MLS*	ALREAGAL
	Gill's Hematoxylin I	American Master Tech Scientific/MLS	HXGHE1LT
	Xylene	Fisher Scientific/MLS	X3P-1GAL
	Tissue-Tek® Vertical 24 Slide Rack	American Master Tech Scientific/MLS	LWSRA24
	Tissue-Tek® Staining Dish (6 required)	American Master Tech Scientific/MLS	LWT4457EA
	Tissue-Tek® Clearing Agent Dish, xylene resistant (3 required)	American Master Tech Scientific/MLS	LWT4456EA
	Cytoseal XYL xylene-based mounting medium	Richard-Allen Scientific/MLS	8312-4
	Cover Glass 24 x 50 mm	Fisher Scientific/MLS	12--545-F

☑	Description	Supplier	Cat. no.
	Ammonium hydroxide, 28–30%	Sigma-Aldrich/MLS	320145-500mL
	Carboy (>3L)	MLS	—
	Water bath or incubator, capable of holding temperature at 40 +/- 1°C	MLS	—
	Pipettors and tips, 1–1000 µL	MLS	—
	Distilled water	MLS	—
	Tubes (various sizes)	MLS	—
	Fume hood	MLS	—
	Graduated cylinder	MLS	—
	Parafilm	MLS	—
	Paper towel or absorbent paper	MLS	—
	Glass beaker (1 or 2 L)	MLS	—
	Hot plate	Fisher Scientific/MLS	11-300-49SHP
	Aluminum foil	MLS	—
	Forceps, large	MLS	—
	Thermometer	MLS	—
	20% bleach	MLS	—
	Microscope and accessories	MLS	—
	10% neutral-buffered formalin (NBF)	MLS	—
	Paraffin wax	MLS	—
	Microtome	MLS	—
	Drying oven, capable of holding temperature at 60 +/- 1°C	MLS	—

* 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® Assay on your samples for the first time, we recommend that you:

- View the video demonstrations available at **www.acdbio.com/support/online-training-videos**.
- 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. Refer to **Chapter 3. Prepare and Pretreat Samples** on page 11 for preparation of FFPE slides. For preparation of other sample types, contact **support@acdbio.com**.
- Follow the recommended pretreatment guidelines for your sample. Refer to **Appendix A. Tissue Pretreatment Recommendations** on page 19 for pretreatment conditions.
- 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.
- Use good laboratory practices and follow all necessary safety procedures. Refer to **Appendix C. Safety** on page 23 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. 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 to **Appendix A. Tissue Pretreatment Recommendations** on page 19.

Prepare FFPE sections

Materials required

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

Fix the sample

1. Remove sample and **CUT 3–4 mm** pieces prior to fixing.

   CAUTION! Handle biological specimens appropriately.

2. Fix sample in **FRESH 10% NBF** for **16–32 HOURS** at **ROOM TEMPERATURE**. Fixation time will vary depending on tissue type.

   IMPORTANT! Under-fixation will result in significant signal loss when performing the RNAscope® 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**.

   Note: Embedded samples may be stored at room temperature for years.

4. Trim paraffin blocks as needed, and **CUT** embedded tissue into **5 +/- 1 µm** sections using a microtome.
5. Place paraffin ribbon in water bath, and **MOUNT** sections on **SUPERFROST® PLUS SLIDES.**

   IMPORTANT! Place sections in the center of the slide.

6. **AIR DRY** slides **OVERNIGHT** at **ROOM TEMPERATURE**. Do **NOT** bake slides unless they will be used within 1 week.

   OPTIONAL STOPPING POINT (1). Use sectioned tissue within 3 months. Store sections with dessicants at room temperature.

Prepare FFPE slides for the RNAscope® Assay

Workflow

Bake slides ~1 hour

↓

(*Optional*) 1 week Stopping Point (2)

↓

Deparaffinize FFPE tissue ~20 minutes

↓

(*Optional*) Overnight Stopping Point (3)

Materials required

- Drying oven
- Prepared FFPE slides
- Tissue-Tek® Vertical 24 Slide Rack
- Distilled water
- Fume hood
- Xylene
- 100% ethanol
- Tissue-Tek® Clearing Agent Dish (2)
- Tissue-Tek® Staining Dish (2)
- ImmEdge™ Hydrophobic Barrier Pen

Bake slides

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

   OPTIONAL STOPPING POINT (2). Use sectioned tissue within 1 week. Store sections with dessicants at room temperature.

2. If you continue, prepare the materials for the following protocols while the slides are baking: Deparaffinize FFPE sections on page 13 and **PRETREAT SAMPLES** on page 15.

Deparaffinize FFPE sections

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

- 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% ETHANOL**.

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 MINUTES** at **ROOM TEMPERATURE**. 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% ethanol.
6. Incubate the slides in **100% ETHANOL** for **1 MINUTE** at **ROOM TEMPERATURE** with agitation.
7. **REPEAT STEP 6** with **FRESH 100% ETHANOL**.
8. Remove the slides from the rack, and place on absorbent paper with the section face-up. **AIR DRY** for **5 MINUTES** at **ROOM TEMPERATURE**.

   OPTIONAL STOPPING POINT (3). Air dry overnight at room temperature (must use within 24 hours) or proceed directly to the next step.

Pretreat samples

IMPORTANT! Before you begin, make sure you know the pretreatment conditions specific to your sample type. Refer to **Appendix A. Tissue Pretreatment Recommendations** on page 19.

Workflow

Equilibrate equipment and prepare 1X Pretreat 2

Turn on HybEZ™ OVEN.

↓

Place a Humidifying Paper in the Humidity Control Tray

↓

Insert covered tray into oven and close the oven door. **WARM** the **TRAY** for **30 MINUTES** at **40°C** before use.

↓

Prepare 1X Pretreat 2 ~10–20 minutes

↓

Apply Pretreat 1 ~10 minutes

↓

Apply Pretreat 2 ~10–30 minutes

↓

Create Hydrophobic barrier

(*Optional*) Overnight Stopping Point (4)

↓

Apply Pretreat 3 ~30–60 minutes

↓

Proceed IMMEDIATELY to the RNAscope® 2.0 Assay*

* Immediately proceed to the RNAscope® Assay Chapter of the appropriate Part 2 User Manual: RNAscope® 2.0 HD Detection Kit – BROWN (Catalog No. 320497), RNAscope® 2.0 HD Detection Kit – RED (Catalog No. 320487), and RNAscope® 2-Plex Detection Kit – Chromogenic (Catalog No. 320494).

Materials required

Materials provided by Advanced Cell Diagnostics	Other materials and equipment
• Pretreat 1 • Pretreat 2 • Pretreat 3	• Prepared slides • Distilled water • Glass beaker (1 or 2 L) • HybEZ™ Humidifying System • Paper towel or absorbent paper • Hot plate, isotemp brand • Aluminum foil • Thermometer • Forceps, large • Thermometer • Tissue Tek® Slide Rack • Tissue Tek® Staining Dish

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 MINUTES** at **40°C** before use.

Prepare 1X Pretreat 2

IMPORTANT! Do not boil 1X Pretreat 2 more than 30 minutes before use.

1. Prepare **700 mL** of **FRESH 1X PRETREAT 2** by adding 630 mL **DISTILLED WATER** to 1 bottle (70 mL) 10X Pretreat 2 solution in a 1 L beaker. Mix well.
2. Place the beaker containing 1X Pretreat 2 on the **HOT PLATE**. Cover the beaker with foil and turn the hot plate on **HIGH** for **10–15 MINUTES**.
3. Once 1X Pretreat 2 reaches boiling, turn the hot plate knob to 100–104°C to maintain uniform boiling.

Apply Pretreat 1

1. Lay deparaffinized slides on the bench and add **~5–8 DROPS** of **PRETREAT 1** to cover the entire section.

   IMPORTANT! Ensure the slides are placed on a leveled surface.

2. Incubate slides for **10 MINUTES** at **ROOM TEMPERATURE**.

3. Remove Pretreat 1 solution from one slide at a time by tapping the slide on absorbent paper, and/or flicking. *Immediately* insert the slide into a Tissue-Tek® Slide Rack submerged in a Tissue-Tek® Staining Dish filled with **DISTILLED WATER**.
4. **WASH** slides in the **DISTILLED WATER** by moving the rack up and down **3–5 TIMES** and **REPEAT** with **FRESH DISTILLED WATER**.

Apply Pretreat 2

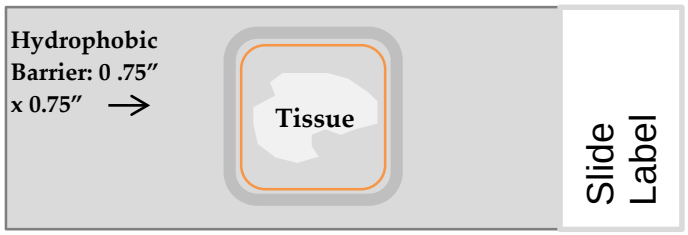
1. Ensure that **1X PRETREAT 2** solution is at mild boiling.
2. With a pair of forceps *very slowly* submerge the slide rack containing the slides into the boiling **1X PRETREAT 2** solution. Cover the beaker with foil and BOIL the slides for the amount of time specified by the table in **Appendix A. Tissue Pretreatment Recommendations** on page 19.
3. After pretreatment time is over, use the forceps to *immediately* transfer the hot slide rack from the **1X PRETREAT 2** to the Staining Dish containing distilled water. Do not let the slides cool in Pretreat 2.
4. WASH slides in the distilled water by moving the rack up and down **3–5 TIMES** and **REPEAT** with **FRESH DISTILLED WATER**.
5. WASH slides in **FRESH 100% ETOH** by moving the rack up and down **3–5 TIMES**. Air dry the slides.

Create a hydrophobic barrier

1. Use the following template to **DRAW** a barrier **2–4 TIMES** around each section with the **IMMEDGE™ HYDROPHOBIC BARRIER PEN**. See example below (size of this hydrophobic barrier is 0.75″ x 0.75″).

   Note: Refer to **Appendix B. Reagent Volume Guidelines** on page 21 to determine the recommended number of drops needed per slide.

IMPORTANT! Do not let the barrier touch the 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 **~ 2 MINUTES** or **OVERNIGHT at** Room Temperature.

   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 (4). Dry slides at room temperature overnight for use the following day (must use within 24 hours) or proceed directly to the next section.

Apply Pretreat 3

1. Place the dried slides on the HybEZ™ Slide Rack, and add **~5 DROPS** of **PRETREAT 3** 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, 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 19.

   Note: **PREPARE RNASCOPE® 2.0** assay materials during this step.
3. Remove the HybEZ™ Humidity Control Tray from the oven. Remove the HybEZ™ Slide Rack from the tray.
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 with distilled water by moving the rack up and down **3–5 TIMES** and **REPEAT** with **FRESH DISTILLED WATER**.

Proceed to the RNAscope® Assay

While slides are incubating, proceed *immediately* to an RNAscope® Assay. Ensure you have the appropriate user manual. User manuals are available at **www.acdbio.com/support/technical-doc**. For any questions, please contact **support@acdbio.com**.

Note: Slides can stay in distilled water for up to 15 minutes.

IMPORTANT! Slides should not stay in distilled water for longer than 15 minutes. Proceed to the RNAscope® Assay using the appropriate user manual.

Troubleshooting

For troubleshooting information, refer to the *Troubleshooting User Manual* (Cat. no. 320519) available at: **www.acdbio.com/support/technical-doc.** For any questions, please contact **support@acdbio.com**.

A Appendix A. Tissue Pretreatment Recommendation

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

- Any new or previously untested FFPE tissue types
- Suboptimally prepared samples.

Tissue pretreatment recommendation

1. Stain representative samples using the positive and negative control probes.
2. Fix sample in **FRESH** 10% **NBF** for **16-32 HOURS** at **ROOM TEMPERATURE**.

   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 (see section below), vary the **PRETREAT 2** and/or **PRETREAT 3 TIME**.

Reagent	Mild	Standard	Extended
Pretreat 2	15 min	15 min	30 min
Pretreat 3	15 min	30 min	30 min

Note: Sample types such as certain Xenografts and Cell Pellets, require less time. For these tissue types, vary the **PRETREAT 2 TIME** to 8 minutes and **PRETREAT 3 TIME** to 15 minutes**.** If you have a tissue type not listed, contact support at **support@acdbio.com.**

Tissue-specific pretreatment conditions

If your sample fixation is successful in fresh 10% NBF (Step 2 above), 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

Species	Tissue type	Pathology	Pretreatment Condition
	Eye/Retina	Normal	Standard
	Liver	Normal	Extended
	Kidney	Normal	Standard
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	Mild
	Lymph node	Normal	Mild
	Tonsil	Normal	Mild
	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)	—	Standard

*Tissue Microarray

B

Appendix B. Reagent Volume Guidelines

Determine reagent volume

Before starting your experiment, measure the inner edge of the hydrophobic barrier to determine the recommended number of drops needed per slide (see table below).

Size of hyrophobic barrier* (in)	Recommended number of drops per slide	Recommended volume per slide (µL)	Relative template size
0.75” x 0.75” †	4	120	
0.75” x 1.0”	5	150	
0.75” x 1.25”	6	180	

* Hydrophobic barrier measured at inner edge. References in this user manual are for the 0.75″ x 0.75″ hydrophobic barrier size.

† Recommended hydrophobic barrier size is 0.75″ x 0.75″. With this barrier size, each probe is sufficient for staining ~20 sections. Larger tissue sections will result in fewer tests.

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 Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain MSDSs, see **Documentation and Support** in this document.
- 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: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/%2029cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov/**

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: **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: **eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:133:0001:0043:EN:PDF**

Documentation and support

Obtaining MSDSs

Material Safety Data Sheets (MSDSs) are available at: **www.acdbio.com/support/technical-doc/category/msds**. For the MSDSs of chemicals not distributed by Advanced Cell Diagnostics, contact the chemical manufacturer.

Obtaining support

For the latest services and support information, go to: **www.acdbio.com/support**

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, MSDSs, application notes, citations, training videos, and other product support documents.
- Find out information about customer training events.

Contact information

Advanced Cell Diagnostics, Inc.

3960 Point Eden Way
Hayward, 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 at **www.acdbio.com/tos/terms-and-conditions-of-sale**. If you have any questions, please contact Advanced Cell Diagnostics at **www.acdbio.com/support**.

Headquarters
3960 Point Eden Way | Hayward, CA 94545 | Phone 1-510-576-8800 | Toll Free 1-877-576-3636
For support, email support@acdbio.com.
www.acdbio.com

ADVANCED CELL DIAGNOSTICS, INC.