USER MANUAL

DNAscope™ HD Duplex Detection Kit

(Chromogenic)

With Sample Preparation and Pretreatment

Document Number UM 324700

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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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Chapter 1. Product Information

Before using this product, read and understand the safety information in **Appendix E. Safety**.

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

About this guide

This user manual provides guidelines and protocols to use the DNAscope HD Duplex Reagent Kit (Cat. No. 324700) on formalin-fixed, paraffin-embedded (FFPE) samples. DNAscope Assays are compatible with a variety of sample types including tissue microarray (TMA) and cell samples.

Visit **www.acdbio.com/technical-support/user-manuals** to download a technical note for other sample types or contact **support.acd@bio-techne.com** for more information.

Product description

Background

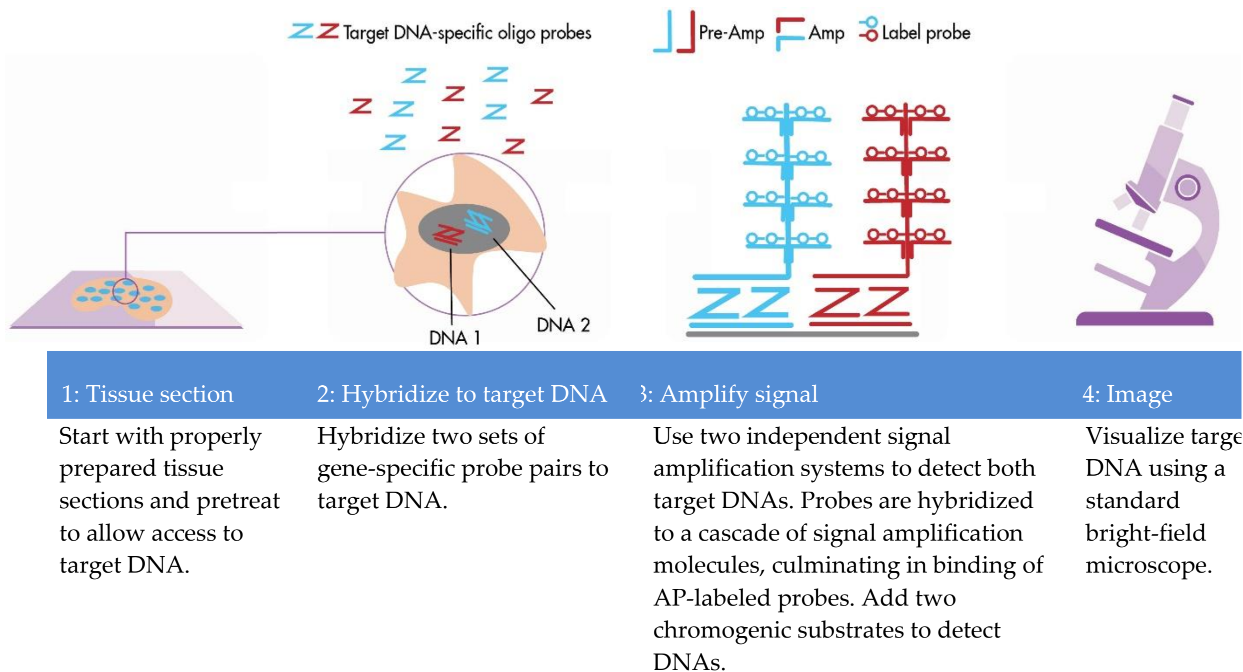
The DNAscope HD Duplex Chromogenic Assay uses a novel and proprietary method of *in situ* hybridization (ISH) to simultaneously visualize two DNA targets in samples mounted on slides. The assay is based on ACD's patented signal amplification and background suppression technology and incorporates multiplexed signal amplification systems, which enable users to investigate expression as well as positional relationship between two different genes within a cellular context.

Overview

The DNAscope HD Duplex Chromogenic Assay procedure can be completed in 24 hours, conveniently divided over two days (see Figure 1). Most of the DNAscope Assay reagents are available in convenient Ready-To-Use (RTU) bottles.

Starting with properly prepared tissue samples, sections are first pretreated, and then DNAscope-specific probes are hybridized to target DNA. The DNAscope HD Duplex Chromogenic Assay employs two independent signal amplification systems, each using the same chromogenic enzyme. Two target genes appear as punctate dots of two distinctly colored chromogen precipitates, visible using a common bright-field microscope at 40–60X magnification.

Figure 1. Procedure overview



Kit contents and storage

The DNAscope HD Duplex Chromogenic Assay requires the DNAscope Probes and the DNAscope Detection Kit. Probes and Detection Kits are available separately.

DNAscope Probes

Visit **https://acdbio.com/products** to find a gene-specific target probe. Each target probe contains a mixture of short oligonucleotides designed to bind to a specific target DNA and is detectable in one of two color channels, C1 or C2.

Probe Channel ID	Chromogenic Labels	
	Enzyme	Color
C1*	AP	RED
C2	AP	BLUE

* Default channel

Channel C1 target probe is available as a Ready-To-Use solution, while the Channel C2 is available as a 50X stock. To independently detect two target DNAs in a duplex assay, each target probe must be in a different color channel and there must be a C1 probe in the mixture.

Each bottle contains enough probe to stain ~20 sections, each with an area of approximately 20 mm x 20 mm (0.75" x 0.75"). Larger tissue sections will result in fewer tests. The probes have a shelf life of two years from the manufacturing date when stored as indicated in the following table:

Target Probes					
☑	Reagent	Cat. No.	Content	Quantity	Storage
	DNAscope Target Probe – *[species]* – *[gene]-C1]*	Various	Ready-To-Use (RTU) probe for color channel C1	3 mL x 1 bottle	2–8 °C
	DNAscope Target Probe – *[species]* – *[gene]*-C2	Various	50X probe for color channel C2	60 µL x 1 tube	2–8 °C

DNAscope HD Duplex Reagent Kit

Each DNAscope HD Duplex Reagent Kit (Cat. No. 324700) provides enough reagents to stain ~20 tissue sections, each with an area of approximately 20 mm x 20 mm (0.75″ x 0.75″). Larger tissue sections will result in fewer tests.

The reagents have a shelf life of nine months from the manufacturing date when stored as indicated in the following tables:

Pretreatment Reagents (Cat. No. 323750)				
☑	Reagent	Cat. No.	Quantity	Storage
	RNA Removal Solution	323748	4.5 ml x 1 bottle	2–8°C
	Hydrogen Peroxide	322335	3 mL x 2 bottles	2–8°C
	Protease Plus	322331	4.5 mL x 1 bottle	2–8°C
	DNAscope Target Retrieval (10X)	323745	70 mL x 4 bottles	Room temp (15–30°C)

DNAscope HD Duplex Detection Kit (Cat. No. 324710)			
☑	Reagent	Quantity	Storage
	DNAscope HD Duplex AMP 1	3 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 2	3 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 3	4.5 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 4	4.5 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 5	4.5 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 6	3 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 7	4.5 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 8	4.5 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 9	4.5 mL x 1 bottle	2–8°C
	DNAscope HD Duplex AMP 10	3 mL x 1 bottle	2–8°C
	DNAscope Fast Red-A — diluent	3 mL x 1 bottle	2–8°C
	DNAscope Fast Red-B — substrate	60 µL x 1 tube	2–8°C
	DNAscope Blue-A — diluent	3 mL x 1 bottle	2–8°C
	DNAscope Blue-B — substrate	60 µL x 1 vial	2–8°C

Wash Buffer Kit (Cat. No. 310091)			
☑	Reagent	Quantity	Storage
	Wash Buffer (50X)	60 mL x 4 bottles	Room temp (15–30°C)

IMPORTANT! DNAscope HD Reagent Kits share the same Wash Buffer, Hydrogen Peroxide, and Protease Plus as other ACD Reagent Kits, but the rest of the components are unique. Do not interchange these reagents between different types of ACD Kits.

Required materials and equipment

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

HybEZ Hybridization System

IMPORTANT! The DNAscope Assay has been developed using this system only.

Use the HybEZ™ Hybridization System to perform DNAscope 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 II Hybridization System User Manual* available at **https://acdbio.com/documents/product-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 II Oven (110 or 220V)	1 oven	321710 or 321720 (HybEZ II)
	HybEZ Humidity Control Tray (with lid)	1 tray	310012
	ACD EZ-Batch™ Slide Holder (20 slide capacity)	1 rack	310017
	HybEZ Humidifying Paper Pack	15 sheets	310015

User-supplied materials

IMPORTANT! Do not substitute other materials for the ImmEdge™ Hydrophobic Barrier Pen or the VectaMount listed in the following table.

☑	Description	Supplier	Cat. No.
	ImmEdge Hydrophobic Barrier Pen (required)	Vector Laboratory	H-4000
	SuperFrost® Plus Slides or SuperFrost Excell Slides	Fisher Scientific	12-550-15 22-034-985
	Gill's Hematoxylin I	American Master Tech Scientific/MLS*	HXGHE1LT
	Xylene	Fisher Scientific/MLS	X3P-1GAL
	100% alcohol (EtOH)	American Master Tech Scientific/MLS*	ALREAGAL
	10% Neutral Buffered Formalin (NBF)/ 4% Paraformaldehyde (PFA) / 37% Formaldehyde	MLS Sigma	— 252549-1L

☑	Description	Supplier	Cat. No.
	1X Phosphate Buffered Saline (PBS)	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 Dish, xylene resistant	American Master Tech Scientific/MLS	LWT4456EA
	VectaMount Permanent Mounting Medium (requir	Vector Labs	H-5000
	Cover Glass, 24 x 50 mm	Fisher Scientific/MLS	12--545-F
	Ammonium hydroxide, 28–30%	Sigma-Aldrich/MLS	320145-500mL
	Carboy (>3L)	MLS	—
	Paraffin wax	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	—
	Microcentrifuge	MLS	—
	Microtome	MLS	—
	Water bath or incubator, capable of holding temperature at 40 +/– 1°C	MLS	—
	Oster® Steamer Model 5712, Black and Decker Steamer HS3000, the Braun Multiquick FS 20 Steamer, or the Hamilton Beach Steamer	—	—
	Microscope and accessories	MLS	—
	Drying oven, capable of holding temperature at 60 +/– 1°C	MLS	—
	Aluminum foil (Optional)†	MLS	—
	Forceps, large (Optional)†	MLS	—
	Hot plate (Optional)†	MLS	—

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

† Required for the alternate target retrieval method in **Appendix C** on page 32.

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Chapter 2. Before You Begin

IMPORTANT! The DNAscope assay has an overnight probe hybridization step. Total assay run time including overnight probe hybridization is ~24 hours.

Prior to running the DNAscope 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**.

Important procedural guidelines

- Start with properly fixed and prepared sections. Refer to **Appendix A. Tissue Pretreatment Recommendation**, **Chapter 3. Prepare and Pretreat Samples,** and pretreatment user guides available at **www.acdbio.com/technical-support/user-manuals**.
- Use only samples mounted on SuperFrost Plus Slides or SuperFrost Excell Slides (Fisher Scientific; Cat. Nos. 12-550-15 and 22-034-985).
- Follow the recommended pretreatment guidelines for your sample. Refer to **Chapter 3. Prepare and Pretreat Samples** and pretreatment user guides available at **www.acdbio.com/technical-support/user-manuals**.
- Do *not* substitute required materials. Assay has been developed with these materials only.
- Follow the protocol exactly for best results.
- Do not let your sections dry out unless it is part of the procedure.
- Use good laboratory practices and follow all necessary safety procedures. Refer to **Appendix E. Safety** for more information.

Chapter 3. Prepare and Pretreat Samples

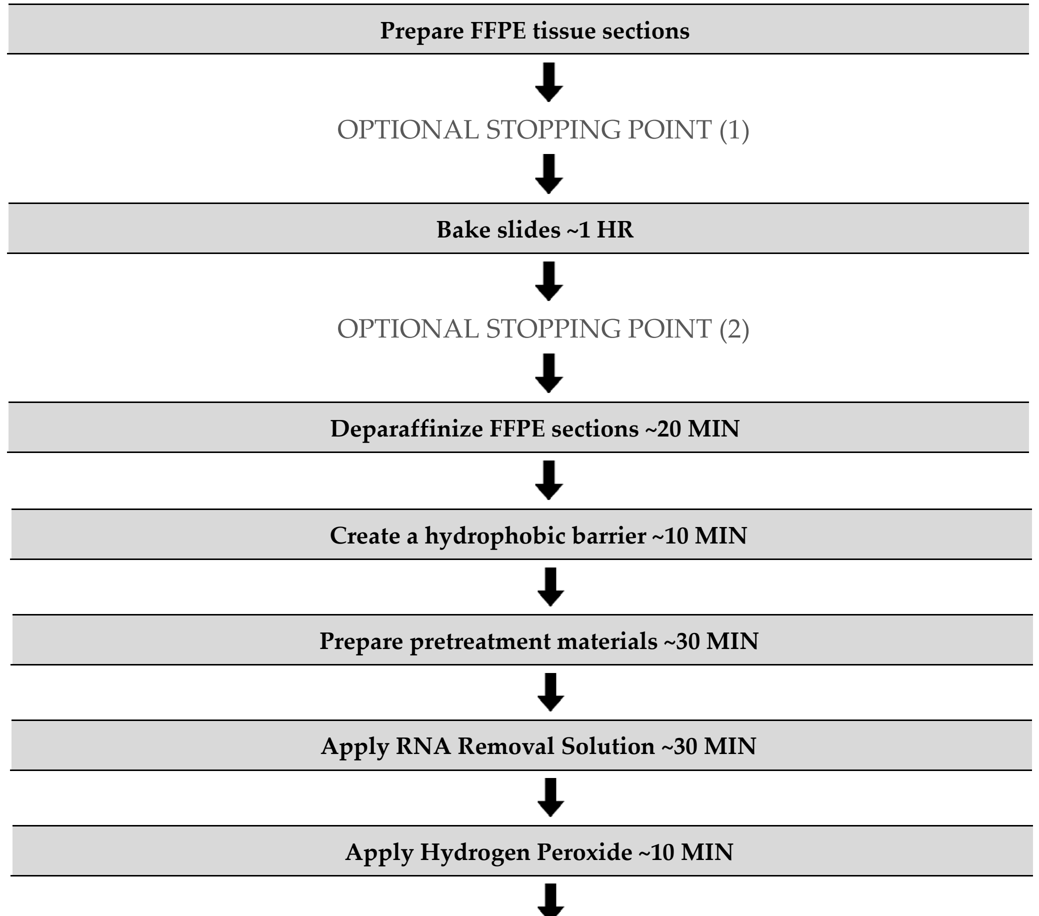
This chapter describes preparation and pretreatment of formalin-fixed, paraffin-embedded (FFPE) samples.

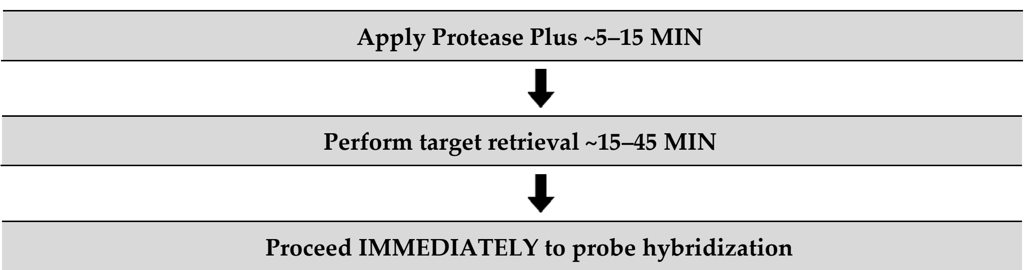
IMPORTANT! We highly recommend following these guidelines. We cannot guarantee assay results with other preparation methods.

FFPE sample preparation and pretreatment

For suboptimally prepared samples, you may need to optimize pretreatment conditions. Refer to **Appendix A. Tissue Pretreatment Recommendation** and to **https://acdbio.com/technical-support/solutions**.

Workflow





Materials required

Materials provided by Pretreatment Reagents (Cat. No. 323750)	Other Materials and Equipment
• RNA Removal Solution • Hydrogen Peroxide • Protease Plus • DNAscope10X Target Retrieval Reagent	• 10% Neutral Buffered Formalin (NBF) or fresh 4% PFA • 1X PBS • Paraffin wax • Tissue-Tek Clearing Agent Dishes • Tissue-Tek Staining Dishes • Tissue-Tek Vertical 24 Slide Rack • 100% alcohol (EtOH) • Xylene • Microtome • Water bath • SuperFrost Plus slides • ImmEdge Hydrophobic Pen • Drying oven • Distilled water • Fume hood • HybEZ Humidifying System/ACD EZ-Batch Slide Holder and Wash Tray • Paper towel or absorbent paper • Steamer • Digital thermometer

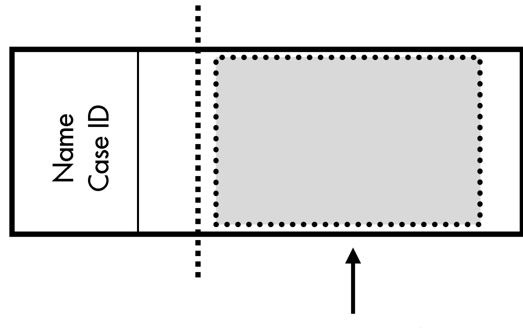
Prepare FFPE tissue sections

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.

IMPORTANT! Fixation for **<16 HRS** or **>32 HRS** will impair the performance of the assay.

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

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



Tissue section location

IMPORTANT! Some tissue specimens are more prone to detachment than others. Please use Superfrost Excell slides for mounting samples if increased tissue detachment is observed.

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

7. Air dry slides **OVERNIGHT** at **RT**.

OPTIONAL STOPPING POINT (1). You can store sections with desiccants at room temperature.

Bake slides

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

OPTIONAL STOPPING POINT (2). Use immediately, or store with desiccants at room temperature.

Note: If you continue with the procedure, you can prepare materials for the next steps while the slides are baking.

Deparaffinize FFPE sections

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

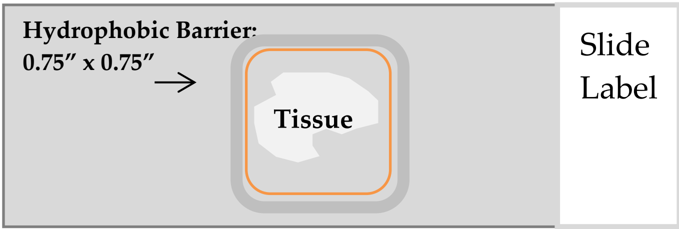
1. In a fume hood:
   - Fill two Tissue-Tek Clearing Agent dishes with ~200 mL fresh xylene.
   - Fill two Tissue-Tek Staining dishes with ~200 mL fresh 100% ethanol.
1. Place slides in a Tissue-Tek Slide Rack and submerge in the first xylene-containing dish in the fume hood.
2. Incubate the slides in xylene for **5 MIN** at **RT**. Agitate the slides by occasionally lifting the slide rack up and down in the dish.
3. Remove the slide rack from the first xylene-containing dish, and *immediately* place in the second xylene-containing dish in the fume hood.
4. Incubate the slides in xylene for **5 MIN** at **RT** with agitation.
5. Remove the slide rack from the second xylene-containing dish, and *immediately* place in a dish containing 100% ethanol.
6. Incubate the slides in 100% ethanol for **2 MIN** at **RT** with agitation.

7. Remove the slide rack from the first ethanol -containing dish, and *immediately* place in the second ethanol -containing dish.
8. Incubate the slides in 100% ethanol for **2 MIN** at **RT** with agitation.
9. Remove the slides from the rack, and place on absorbent paper with the section face-up. Dry slides in a drying oven for **5 MIN** at **60°C** (or until completely dry)**.**

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. Other pens may cause suboptimal results.



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 ~**10 MIN**.

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.

Prepare the materials for day one

Prepare 1X Wash Buffer

- Prepare 3 L of 1X Wash Buffer by adding 2.94 L distilled water and 1 bottle (60 mL) of Wash Buffer (50X) to a large carboy. Mix well.

Note: Warm 50X Wash Buffer up to **40°C** for **10–20 MIN** before preparation. 1X Wash Buffer may be prepared ahead of time and stored at room temperature for up to one month.

Prepare probes

1. Warm probes for at least **10 MIN** at **40°C** in a water bath or incubator.
2. Briefly spin the C2 probe to collect the liquid at the bottom of the tubes.
3. Mix 1:50 ratio of C2 probe to C1 probe by pipetting 1 volume of C2 probe to 50 volumes of C1 probe into a tube. Invert the tube several times.Warm probes for at least **10 MIN** at **40°C** in a water bath or incubator.

Note: Do not mix probes of the same color channel. The mixed target probes can be stored at **4°C** for up to six months.

Prepare pretreatment materials

1. Turn on the HybEZ Oven, and set the temperature to **40°C**.
2. Place a Humidifying Paper in the Humidity Control Tray and wet completely with distilled water.

3. Insert the covered tray into the 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.
4. Prepare 1X DNAscope Target Retrieval Reagents (for example, add 180 mL distilled water to 20 mL 10X DNAscope Target Retrieval Reagents). Mix well.

Apply DNAscope RNA Removal Solution

1. Load the dry slides into the ACD EZ-Batch Slide Holder by opening the swing clamp (see page 33 for detailed instructions).
2. Add ~5–8 drops of of DNAscope RNA removal solution to each slide. Make sure each section is completely covered.
3. Place the ACD EZ-Batch Slide Holder in the pre-warmed HybEZ Humidity Control Tray. Close the lid, seal, and insert the tray back into the oven.
4. Incubate for **30 MIN** at **40°C**.
5. Pour at least 200 mL distilled water into the transparent ACD EZ-Batch Wash Tray.
6. Remove the HybEZ Humidity Control Tray from the oven. Remove the slide holder from the tray. Place the tray back into the oven.
7. Place the ACD EZ-Batch Slide Holder into the wash tray containing water. Make sure all the slides are submerged. If needed, carefully add more water. Wash the slides with slight agitation (see page 34 for details).
8. Repeat the wash step with fresh distilled water.

Apply Hydrogen Peroxide

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder, and add ~5–8 drops of Hydrogen Peroxide to cover each section.
2. Incubate slides for **10 MIN** at **RT**.
3. Pour at least 200 mL distilled water into the transparent ACD EZ-Batch Wash Tray.
4. Place the ACD EZ-Batch Slide Holder into the wash tray containing water. Make sure all the slides are submerged. If needed, carefully add more water. Wash the slides with slight agitation.
5. Repeat the wash step with fresh distilled water.

Apply Protease Plus

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder, and add ~5 drops of Protease Plus to entirely cover each section.
2. Place the ACD EZ-Batch Slide Holder in the pre-warmed HybEZ Humidity Control Tray for incubation at **40°C** or on the bench for incubation at **RT** depending on your sample (see **Appendix A. Tissue Pretreatment Recommendation** on page 31).

Note: If needed, prepare DNAscope Assay materials during this step.

3. Pour at least 200 mL distilled water into the transparent ACD EZ-Batch Wash Tray.
4. Remove the HybEZ Humidity Control Tray from the oven if you were incubating the slides at **40°C**. Remove the slide holder from the tray. Place the tray back into the oven.
5. Place the ACD EZ-Batch Slide Holder into the clear plastic wash tray containing water. Make sure all the slides are submerged. If needed, carefully add more water. Wash the slides with slight agitation.
6. Repeat the wash step with fresh distilled water.

Perform target retrieval using a steamer

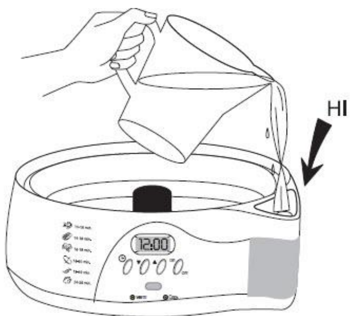
IMPORTANT! Before you begin, make sure you know the pretreatment conditions specific to your sample type from **Appendix A. Tissue Pretreatment Recommendation**.

We highly recommend using a steamer for target retrieval. For target retrieval using a hot plate, see **Appendix C. Manual Target Retrieval** on page 32.

Note: For each steamer, fill the water to the maximum level before starting and do not refill water during the steaming process. Refilling water during steaming process will drop the temperature and interfere with Target Retrieval.

1. Fill the water reservoir with cold tap water to the MAX fill marking line.

IMPORTANT! Do not overfill.



2. Place two slide holders in the steam bowl. Fill one slide holder with 200 mL of 1X DNAscope Target Retrieval Reagent. Fill the other slide holder with 200 mL of distilled H_20.
3. Turn on the steamer. Set the heating time to the maximum so steamer does not shut off during Target Retrieval.
4. Insert a digital thermometer through the holes of the lid and into the container containing 1X DNAscope Target Retrieval Reagent. Allow the temperature to rise to at least **99°C**.
5. Add the slides to the container containing distilled H_20 for **10 SEC** to acclimate the slides.
6. Remove the slides and move them to the container containing1X DNAscope Target Retrieval Reagent. Cover the steamer with the lid.
7. Start the timer for **10–15 MIN** for mild conditions and **30–45 MIN** for standard conditions. For pretreatment times, consult **Appendix A. Tissue Pretreatment Recommendation**.
8. Remove the slides from the target retrieval reagent and transfer to the container with hot distilled water (**100°C**). Allow slides to rinse for **5–10 SEC**.
9. Transfer the slides to a separate container filled with 1X Wash buffer and keep the slides immersed in wash buffer at **RT** until the DNAscope probes are added. Add the probes as quickly as possible.

Proceed to the DNAscope Assay

Proceed *immediately* to **Chapter 4. DNAscope HD Duplex Assay**.

4

Chapter 4. DNAscope HD Duplex Assay

This procedure flows directly from sample preparation and pretreatment. Refer to **Chapter 3** and pretreatment user guides available at **www.acdbio.com/technical-support/user-manuals**.

Workflow

Prepare the materials ~10–30 MIN

⬇

Run the assay ~10 HRS

Hybridize probe overnight ~15–18 HRS

⬇

Hybridize Amp 1 ~30 MIN

⬇

Hybridize Amp 2 ~30 MIN

⬇

Hybridize Amp 3 ~15 MIN

⬇

Hybridize Amp 4 ~30 MIN

⬇

Hybridize Amp 5 ~15 MIN

⬇

Detect the red signal ~10 MIN

⬇

Hybridize Amp 6 ~15 MIN

⬇

Hybridize Amp 7 ~15 MIN

⬇

Hybridize Amp 8 ~30 MIN

⬇

Hybridize Amp 9 ~15 MIN

⬇

Hybridize Amp 10 ~15 MIN

⬇

Detect the blue signal ~10 MIN

⬇

Counterstain the slides ~2 MIN

⬇

Bake the slides ~15 MIN

⬇

Mount samples ~5 MIN

⬇

Evaluate the samples

Materials required for the assay

Materials provided by DNAscope HD Duplex Detection Kit	Materials provided by DNAscope Probes	Other Materials and Equipment
• DNAscope Wash Buffer (50X)	• C1 Target Probe	• Prepared sections
• DNAscope HD Duplex Amp 1	• 50X C2 Target Probe	• Distilled water
• DNAscope HD Duplex Amp 2		• Carboy (>3L)
• DNAscope HD Duplex Amp 3		• Fume hood
• DNAscope HD Duplex Amp 4		• Xylene
• DNAscope HD Duplex Amp 5		• 100% alcohol (EtOH)
• DNAscope HD Duplex Amp 6		• Tissue-Tek Staining Dishes
• DNAscope HD Duplex Amp 7		• Tissue-Tek Clearing Agent Dishes, xylene-resistant
• DNAscope HD Duplex Amp 8		• Gill's Hematoxylin I
• DNAscope HD Duplex Amp 9		• Ammonium hydroxide, 28–30%
• DNAscope HD Duplex Amp 10		• Graduated cylinder
• DNAscope Fast Red-A		• Parafilm
• DNAscope Fast Red -B		• HybEZ Humidifying System/ ACD EZ-Batch Slide Holder and Wash Tray
• DNAscope Blue-A		• Water bath or incubator
• DNAscope Blue-B		• Tissue-Tek Vertical 24 Slide Rack
		• Tubes (various sizes)
		• Paper towel or absorbent paper
		• Pipettors and tips, 1–1000 µL
		• VectaMount
		• Cover Glass, 24 mm x 50 mm
		• Dry oven

Prepare the materials

Some of the materials may be prepared in advance and stored at room temperature.

Prepare the materials for day two

Prepare counterstaining reagents

1. In the fume hood, prepare 50% Hematoxylin staining solution by adding 100 mL Gill's Hematoxylin I to 100 mL distilled water in a staining dish.

Note: 50% Hematoxylin staining solution can be reused for up to 1 week.

2. In the fume hood, prepare 0.02% (w/v) Ammonia water (bluing reagent) by adding 1.43 mL of 1N Ammonium Hydroxide to 250 mL distilled water in a graduated cylinder or other container.
3. Seal the cylinder with parafilm. Mix well 3–5 times.

Note: For assay quantitation, you must use Ammonium Hydroxide.

Prepare mounting reagents

IMPORTANT! Do not reuse deparaffinization reagents to dehydrate the slides after the assay.

- In the fume hood, add ~200 mL xylene to a clearing agent dish.

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

Equilibrate reagents

- Remove Amp 1–10 reagents from refrigerator and place at **RT**.
- Ensure HybEZ Oven and prepared Humidity Control Tray are at **40°C**.

Run the assay

IMPORTANT! Do not let sections dry out between incubation steps. Work quickly and fill barrier with solutions.

IMPORTANT! View the wash step video at **https://acdbio.com/technical-support/learn-more** before proceeding.

Hybridize probes overnight

IMPORTANT! Ensure probes are prewarmed to dissolve any precipitation prior to use.

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add ~4 drops of the probe mixture to entirely cover each slide.

Note: Refer to **Appendix C. Reagent Volume Guidelines** on page 31 to determine the recommended number of drops needed per slide. For example, add 4 drops of the appropriate probe to a 0.75″ x 0.75″ barrier.

3. Close the tray and insert into the HybEZ Oven for **15–18 HRS** at **40°C**.
4. Next day pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
5. Remove the HybEZ Humidity Control Tray from the oven. Remove the slide holder from the tray. Place the tray back into the oven.
6. Place the ACD EZ-Batch Slide Holder into the wash tray containing 1X Wash Buffer. Make sure all the slides are submerged. If needed, carefully add more buffer. Wash the slides with slight agitation for **2 MIN** at **RT** (see page 33 for details). Repeat the wash step with fresh 1X Wash Buffer.

Hybridize Amp 1

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 1 to cover each section.
3. Close the tray and insert into the HybEZ Oven for **30 MIN** at **40°C**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
5. Remove the HybEZ Humidity Control Tray from the oven. Remove the slide holder from the tray. Place the tray back into the oven.

6. Place the ACD EZ-Batch Slide Holder into the wash tray, and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 2

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 2 to cover each section.
3. Close the tray and insert into the HybEZ Oven for **30 MIN** at **40°C**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
5. Remove the HybEZ Humidity Control Tray from the oven. Remove the slide holder from the tray. Place the tray back into the oven.
4. Place the ACD EZ-Batch Slide Holder into the wash tray, and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 3

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 3 to cover each section.
3. Close the tray and insert into the HybEZ Oven for **15 MIN** at **40°C**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
5. Remove the HybEZ Humidity Control Tray from the oven. Remove the slide holder from the tray. Keep the tray at **RT**.
6. Place the ACD EZ-Batch Slide Holder into the wash tray, and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 4

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 4 to cover each section.
3. Close the tray and incubate for **30 MIN** at **RT**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
5. Remove the ACD EZ-Batch Slide Holder from the HybEZ Humidity Control Tray.
6. Place the ACD EZ-Batch Slide Holder into the wash tray, and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 5

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 5 to cover each section.
3. Close the tray and incubate for **15 MIN** at **RT**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
5. Remove the ACD EZ-Batch Slide Holder from the HybEZ Humidity Control Tray.
6. Place the ACD EZ-Batch Slide Holder into the wash tray and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Detect the red signal

1. Briefly spin down the contents of the Red-B tube to be sure that the contents are at the bottom of the tube before opening the cap.
2. Depending on the size of your hydrophobic barrier, make RED working solution per section by using a 1:50 ratio of Red-B to Red -A. For example, for a 0.75″ x 0.75″ barrier, add 3 µL of Red-B to 150 µL of Red-A into a tube. Mix well.

IMPORTANT! Use the RED solution immediately. Do not expose to direct sunlight or UV light.

3. Remove excess liquid from the slides while keeping them locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
4. Pipette ~150 µL RED solution onto each tissue section. Ensure that the sections are covered.
5. Close the tray and incubate for **10 MIN** at **RT**.
6. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
7. Remove the ACD EZ-Batch Slide Holder from the HybEZ Humidity Control Tray, and place the tray back into the oven.
8. Place the ACD EZ-Batch Slide Holder into the wash tray, and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 6

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder.
2. Remove the HybEZ Humidity Control Tray from the oven, and insert the slide holder into the tray.
3. Add enough AMP 6 to cover each section.
4. Close the tray and incubate for **15 MIN** at **40°C**.
5. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
6. Remove the HybEZ Humidity Control Tray from the oven. Remove the slide holder from the tray. Place the tray back into the oven.
7. Place the ACD EZ-Batch Slide Holder into the wash tray and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 7

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 7 to cover each section.
3. Close the tray and insert into the HybEZ Oven for **15 MIN** at **40°C**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray
5. Remove the HybEZ Humidity Control Tray from the oven. Remove the slide holder from the tray.

IMPORTANT! Do not insert the tray into the HybEZ Oven for the rest of the procedure.

6. Place the ACD EZ-Batch Slide Holder into the wash tray, and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 8

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 8 to cover each section.
3. Close the tray and incubate for **30 MIN** at **RT**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
5. Remove the ACD EZ-Batch Slide Holder from the HybEZ Humidity Control Tray.
6. Place the ACD EZ-Batch Slide Holder into the wash tray and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 9

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 9 to cover each section.
3. Close the tray and incubate for **15 MIN** at **RT**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
5. Remove the ACD EZ-Batch Slide Holder from the HybEZ Humidity Control Tray.
6. Place the ACD EZ-Batch Slide Holder into the wash tray and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Hybridize Amp 10

1. Remove excess liquid from the slides while keeping the slides locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
2. Add enough AMP 10 to cover each section.
3. Close the tray and incubate for **15 MIN** at **RT**.
4. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray
5. Remove the ACD EZ-Batch Slide Holder from the HybEZ Humidity Control Tray.
6. Place the ACD EZ-Batch Slide Holder into the wash tray and wash the slides for **2 MIN** at **RT** with slight agitation. Repeat the wash step with fresh buffer.

Detect the blue signal

1. Briefly spin down the contents of the Blue-B tube to be sure that the contents are at the bottom of the tube before opening the cap.
2. Depending on the size of your hydrophobic barrier, make BLUE working solution per section by using a 1:50 ratio of Blue-B to Blue-A. For example, for a 0.75″ x 0.75″ barrier, add 3 µL of Blue-B to 150 µL of Blue-A into a tube. Mix well.

IMPORTANT! Use the BLUE solution immediately.

3. Remove excess liquid from the slides while keeping them locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray.
4. Pipette **~150 µL** BLUE solution onto each tissue section. Ensure that the sections are covered.
5. Close the tray and incubate for **10 MIN** at **RT**.
6. Pour at least 200 mL 1X Wash Buffer into the transparent ACD EZ-Batch Wash Tray.
7. Remove the ACD EZ-Batch Slide Holder from the HybEZ Humidity Control Tray.

8. Place the ACD EZ-Batch Slide Holder into the wash tray and wash the slides for **5 MIN** at **RT** with slight agitation.
9. Rinse the slides quickly in water to remove excess wash buffer.

Counterstain the slides

1. Move the Tissue-Tek Slide Rack into the staining dish containing 50% Hematoxylin staining solution for **2 MIN** at **RT**. Slides will turn purple.
2. Transfer the slide rack into a staining dish filled with tap water. Repeat wash step once or twice until there is no remaining hematoxylin left.
3. Replace tap water in the staining dish with 0.02% ammonia water. Move the rack up and down five times. Sections should turn blue.
4. Replace ammonia water with tap water and wash the slides 3–5 times.

Mount the samples

1. Remove the slide rack from the staining dish and dry slides in a **60°C** dry oven for **10–15 MIN**. Avoid prolonged dehydration.
2. Cool the slides for **5 MIN** at **RT**.

IMPORTANT! We do not recommend dehydrating the slides in alcohol.

IMPORTANT! The BLUE substrate is partially soluble in xylene. If using xylene-based automated coversliping, minimize xylene exposure by using one rack at a time.

IMPORTANT! Use the VectaMount mounting medium for manual coverslipping.

3. Briefly dip one slide into fresh pure xylene and immediately place 1–2 drops of VectaMount on the slide before the xylene dries.
4. Carefully place a 24 mm x 50 mm coverslip over the tissue section. Avoid trapping air bubbles.
5. Repeat steps 3 and 4 for each slide.
6. Air dry slides for ≥**5 MIN**.

Evaluate the samples

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

- Assess tissue and cell morphology.
- Assess signal strength. Signal should be visible as punctuate dots within the cell nucleus at 40–60X magnification.

Troubleshooting

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

Chapter 5. Scoring Guidelines

Intended use

DNAscope is intended for research use only (RUO). The following scoring guidelines are recommended as a starting point for data interpretation of gene amplification, gene deletion and gene rearrangement. Criteria for scoring individual cells as positive or negative and for scoring tissue samples as positive or negative for the presence of a specific genomic alteration should be determined by the end-user for their specific application(s) through appropriate validation studies.

Microscope objective recommendation

For identifying the relevant tissue/regions for scoring, a 40x objective is recommended.

Assessable tissue

- Tissue specimen should be evaluated by a pathologist to identify relevant tumor areas to be scored.
- Locate tumor cells on H&E-Stained slide if needed. Evaluate the same area on the DNAscope test slide.
- Scan several areas of the tumor to account for possible heterogeneity.
- Avoid areas with dense inflammation, necrosis, and poorly defined nuclear boundaries.
- Disregard nuclei with weak signal intensity and/or non-specific (high background) staining.
- Do not score nuclei demonstrating evidence of over-digestion (poorly defined nuclear borders and suboptimal morphology).
- Adjust microscope focus to locate all signals in the individual nuclei.

Signal enumeration for gene amplification

- A minimum of 100 nuclei from representative (tumor) cells should be scored per sample.
- Count the number of dots for the target gene (red) and chromosome enumeration probe (CEP) (blue) in representative tumor areas. (See counting guide.)
- Calculate the amplification ratio by dividing the total number of red (target gene) dots in each cell by the total number of blue (CEP) dots in that cell.
- Nuclei with high levels of target gene (red) amplification may exhibit formation of signal clusters. In such nuclei, estimate the number of red dots.
- Nuclei exhibiting dots of only one color should not be scored.
- The ratio of red: blue dots in samples that lack target gene amplification is expected to be <2, and a red: blue dots ratio ≥ 2 or large clusters of red signals indicate gene amplification.

Gene amplification counting guide

Figure 2. Example of ERBB2 amplification in DNAscope assay

Target

CEP17

ERBB2

CEN17

Chr 17

ERBB2

ERBB2 amplified

Two blue signal indicates the presence of two copies of chromosome 17. Two red signal indicates the presence of two copies of ERBB2 gene.
The ratio of ERBB2/CEP17 is 2/2 = 1; non amplified

Two blue signal indicates the presence of two copies of chromosome 17. Six red signal indicates the presence of 6 copies of ERBB2 gene.
The ratio of ERBB2/CEP17 is 6/2 =3 amplified

Do not score nuclei with only blue signal.

Do not score nuclei with only red signal.

Signal enumeration for gene deletion

- A minimum of 100 nuclei from representative (tumor) cells should be scored per sample.

- Count the number of dots for the target gene (red) and chromosome enumeration probe (CEP) (blue) in representative (tumor) areas. (See counting guide.)
- A partial loss or complete loss of red signals compared to blue signals indicates gene deletion.

Gene deletion counting guide

Figure 3. Example of CDKN2A deletion in DNAscope assay

CEP9
Target
CEN9
CDKN2A
Chr 9

CDKN2A deleted
CDKN2A wildtype

In nuclei with no gene deletion, there are two blue signals and two red signals indicating the presence of two copies of CEP9 chromosome and CDKN2A.

In nuclei with homozygous deletion of CDKN2A, there is loss of all red signal and two blue signal for the CEP9 chromosome.

In some nuclei with homozygous deletion of CDKN2A, there is loss of all red signal and one blue signal for the CEP9 chromosome.

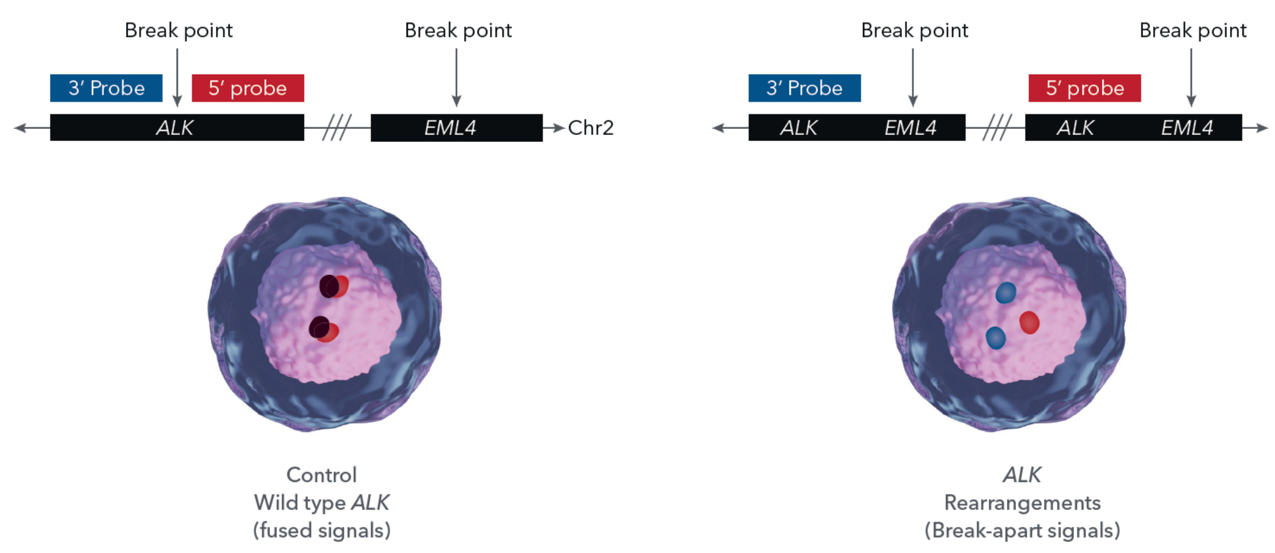
Signal enumeration for gene rearrangement

- A minimum of 100 nuclei from representative (tumor) cells should be scored per sample.
- The DNAscope break-apart probes enable detection of gene rearrangements through the appearance of pure blue signals. (See counting guide.)
- In nuclei with no rearrangement, red and blue signals will overlap and typically, the two overlapping colors are dominated by the red color and appear dark red. It is not necessary to distinguish between dark red and pure red signals to determine break-apart events (see below).

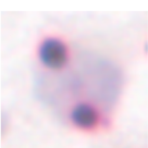
- In nuclei harboring a rearrangement, blue and red signals will be separated, or the red signal will be absent, and **<u>the appearance of a pure blue dot in a nucleus indicates gene break apart.</u>**

Gene rearrangement counting guide

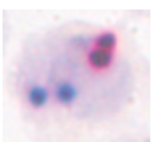
Figure 4. Example of gene rearrangement in DNAscope assay



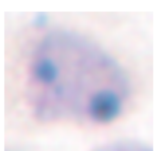
BREAK-APART EVENTS ARE DETECTED THROUGH APPEARANCE OF PURE BLUE DOTS



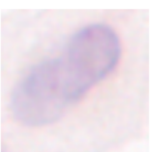
In nuclei with no rearrangement, blue and red signals will overlap. Typically, a dark red/purple signal is observed in these cases



In nuclei with rearrangement, blue and red signals will separate. Appearance of a pure blue dot is observed in these cases, indicating gene rearrangement.



In some nuclei with rearrangement, blue and red signals will separate. In this case, the red signal is lost indicating rearrangements.



Do not score. Nuclei are overlapping and the signal is weak.

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 **Chapter 3**.

Tissue pretreatment recommendation

1. 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 DNAscope Assay.

2. Depending on your tissue type vary the amount of time for the Target Retrieval Reagents and/or Protease Plus.

Reagent	Mild	Standard
Target Retrieval	10–15 MIN	30–45 MIN
Protease Plus	0–5 MIN at RT	15 MIN at 40°C

Note: Sample types, such as certain xenografts and cell pellets, require less time. For these tissue types, vary the Protease Plus time to 0-5 MIN depending on the fixation condition, and the Target Retrieval time to **15 MIN.** If you have a tissue type not listed, contact support at **support.acd@bio-techne.com.**

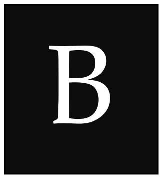
Tissue-specific pretreatment conditions

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.acd@bio-techne.com.**

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
	Pancreas	Normal	Standard
	Cervical	Cancer	Standard

Species	Tissue Type	Pathology	Pretreatment Condition
	Cervical	Normal	Standard
	Brain	Tumor	Standard
	Brain	Normal	Standard
	Head	Cancer	Standard
	Neck	Cancer	Standard
	Liver	Cancer	Standard
	Kidney	Normal	Standard
	Skin	Normal	Standard
	Skin (TMA*)	Normal	Standard
	Breast (TMA)	Normal	Standard
	Stomach (TMA)	Normal	Standard
	Stomach (TMA)	Tumor	Standard
	HeLa (ACD controls) or Cell pellets, fixed with 10% NBF or 10% Formaldehyde	—	Mild

* Tissue Microarray



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.

Size of Hydrophobic 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.

Appendix C. Manual Target Retrieval

Materials required

Materials provided by the Pretreatment Reagents Kit	Other Materials and Equipment
• 10X DNAscope 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 Target Retrieval Reagents

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

1. Prepare 700 mL of fresh1X DNAscope Target Retrieval Reagents by adding 630 mL distilled water to 1 bottle (70 mL) 10X DNAscope Target Retrieval Reagents in the beaker. Mix well.
2. Place the beaker containing1X DNAscope 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 the 1X DNAscope Target Retrieval Reagents reach 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 Target Retrieval Reagents

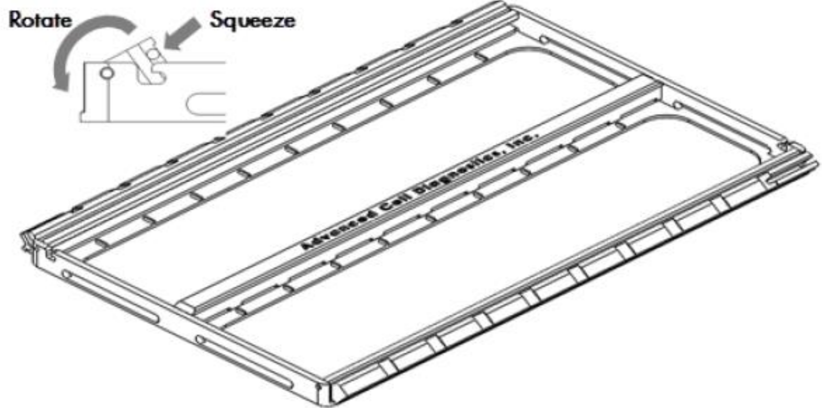
1. With a pair of forceps *very slowly* submerge the slide rack containing the slides into the mildly boiling 1X DNAscope Target Retrieval Reagents solution. Cover the beaker with foil, and boil the slides for the amount of time specified by the table in **Appendix A. Tissue Pretreatment Recommendation** on page 31.
2. Use the forceps to *immediately* transfer the hot slide rack from the1X DNAscope Target Retrieval Reagents to the staining dish containing hot distilled water at **100°C**. 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 hot distilled water.
4. Store slides in 1X Wash Buffer and immediately add probes.

D Appendix D. Using the EZ-Batch Slide Holder and Wash Tray

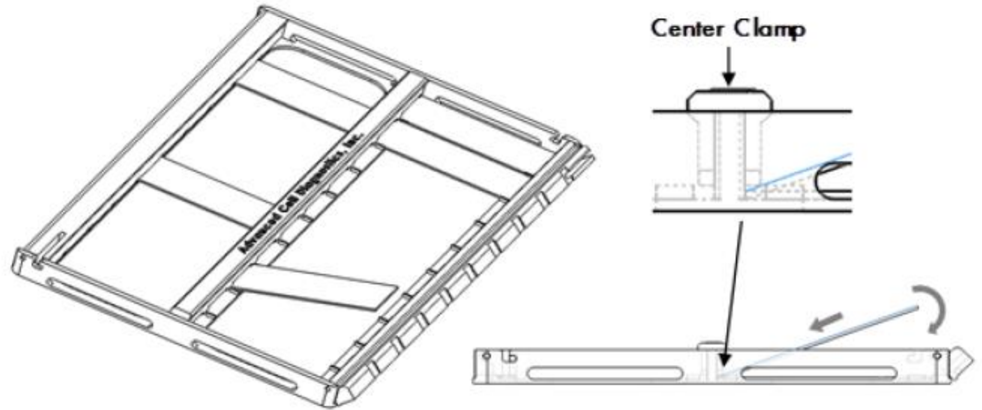
Load the slides in the ACD EZ-Batch Slide Holder

The ACD EZ-Batch Slide Holder can hold up to 20 standard glass slides in secure, lock-down positions arranged in two parallel columns. Lock-down is achieved by two lockable swing clamps, one per column, along both sides of the slide holder. Clamp locking mechanisms are located at the slots found at one end of each clamp.

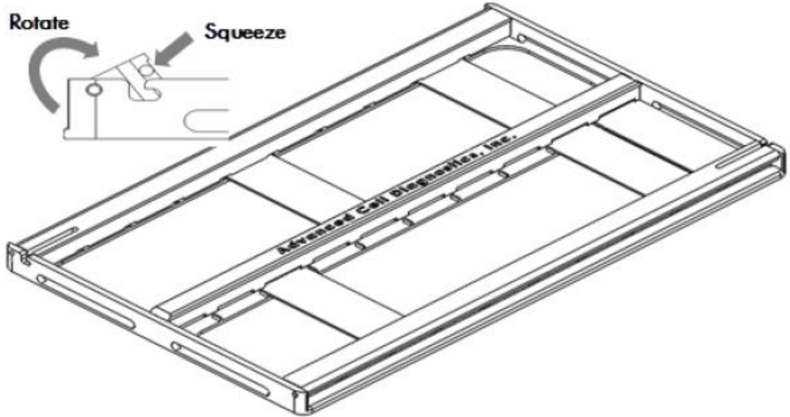
1. Open the swing clamps one at a time by simultaneously squeezing (pressing and holding) the slotted portion of each clamp and rotating it up then outwards, as shown.



2. Insert the slides one at a time into the holder (up to 10 slides per column). The non-label end of each slide should be aligned toward the center of the holder and inserted under the fixed clamp, as shown. Place the rest of the slide down into the holder.

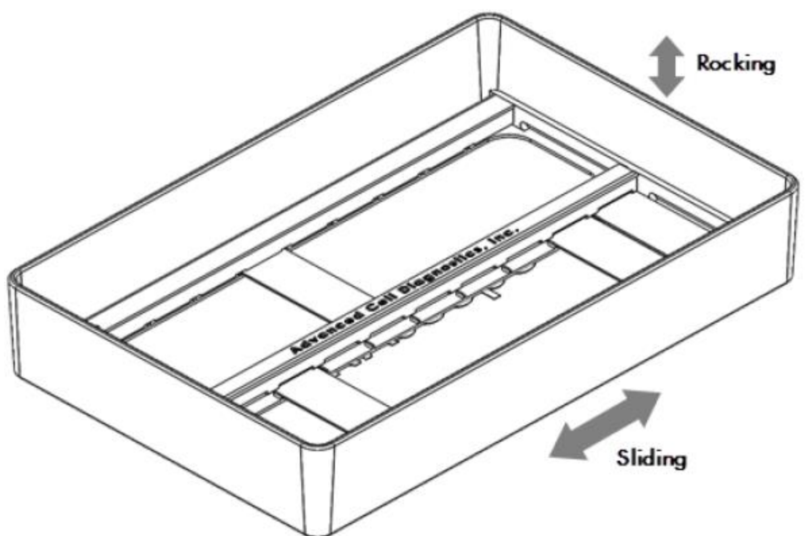


3. Close and lock the swing clamp of the column by simultaneously squeezing the slotted portion of each clamp and rotating it in then downwards in the direction opposite to the direction used to open the clamp, as shown.



Wash slides in the Wash Tray

1. Place the ACD EZ-Batch Slide Holder into the clear plastic wash tray cotaining water. Make sure all the slides are submerged. If needed, carefully add more water. Wash the slides with slight agitation.



Appendix E. 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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7707 Gateway Blvd Suite 200

Newark, CA 94545

Toll Free: 1-877-576-3636

Direct: 1-510-576-8800

Fax: 1-510-576-8801

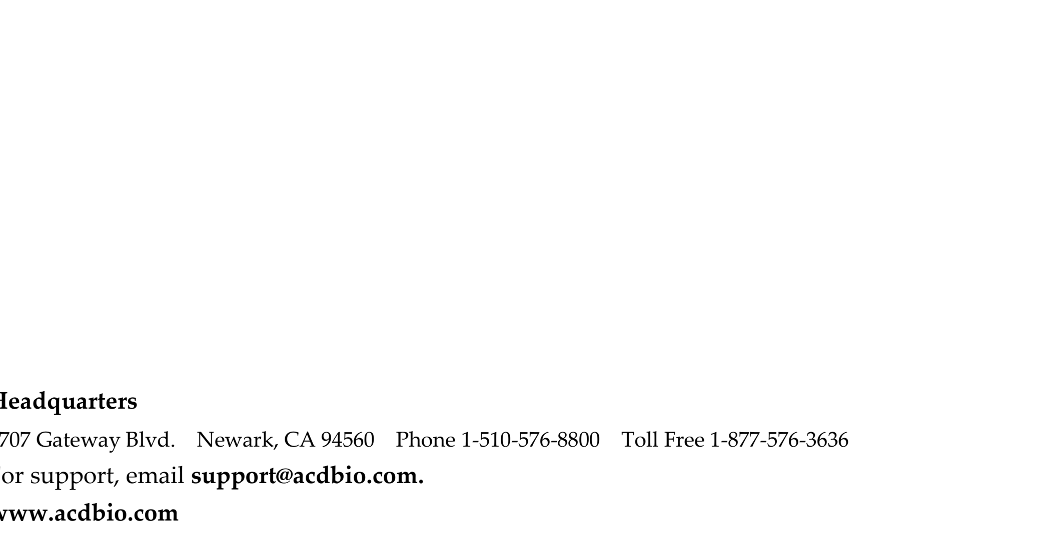
Information: **info.acd@bio-techne.com**

Orders: **orders.acd@bio-techne.com**

Support Email: **support.acd@bio-techne.com**

Limited product warranty

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



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For support, email **support@acdbio.com.**

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