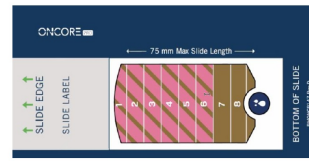


HCR RNA-ISH Setup Oncore Kit



HCR RNA-ISH Setup Oncore Kit Instructions

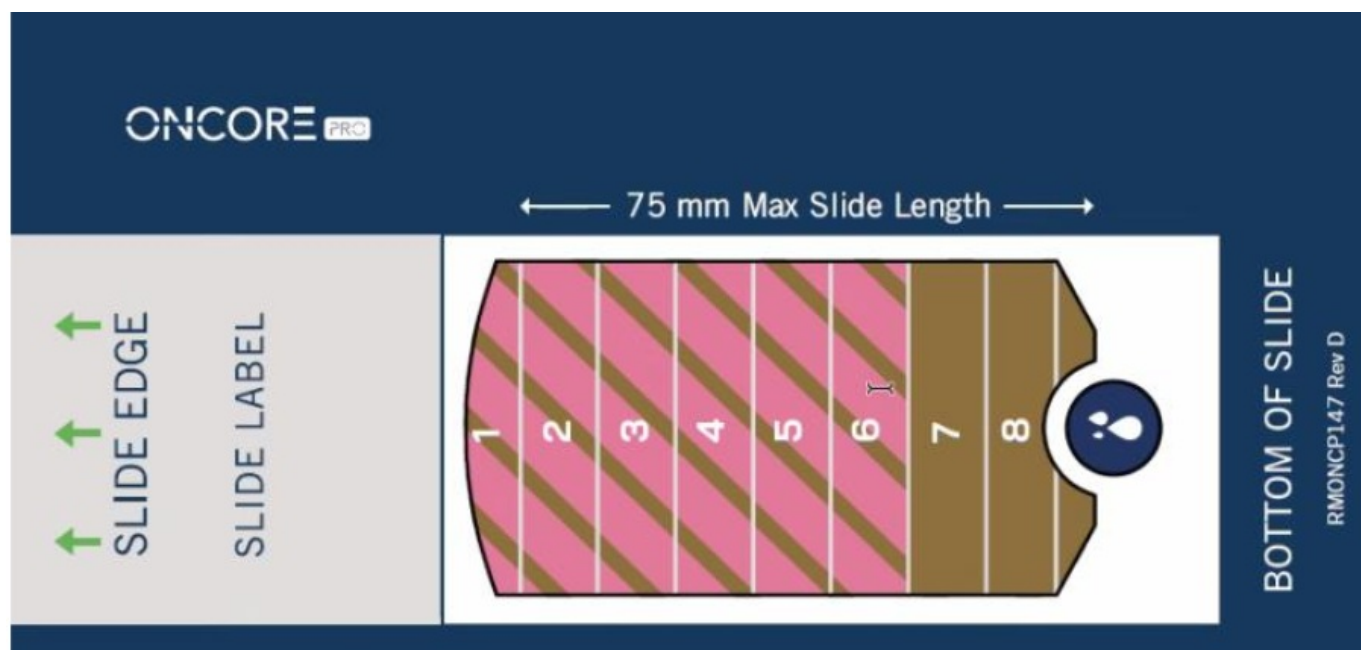
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HCR™
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HCR RNA-ISH Setup Oncore Kit



Specifications

- **Product Name:** HCRTM RNA-ISH Kit
- **Compatibility:** ONCORE Pro X
- **Last Updated:** 01/08/2024

Product Information

Upon receiving an HCRTM RNA-ISH Kit, please check all reagents and their storage conditions.

HCRTM RNA-ISH Starter Kit

HCRTM Reagents	Amount	Storage Temperature
HCRTM Probe A Diluent	1 mL	–
HCRTM Probe B Diluent	1 mL	–

Product Usage Instructions

Reagent Preparation

Follow these steps for preparing the probe solutions:

1. Mix HCRTM Probe A Diluent with the provided volume of HCRTM Probe A.
2. Repeat the same process for HCRTM Probe B Diluent and HCRTM Probe B.

Preparation of Probe Solutions

Estimate the required probe solutions based on the assay needs and proceed with preparation.

Reagent Transfer and Storage

Ensure proper transfer of reagents and store them according to the specified storage conditions.

Overall Workflow of the HCRTM RNA-ISH + IHC/IF Protocol

Follow the specific protocol outlined in the manual for combined detection assays.

FAQ

- **How should I store the HCRTM RNA-ISH Kit reagents?**

Store the reagents at the specified temperatures as indicated in the product information section of the manual.

- **Can I use the HCRTM RNA-ISH Kit with other equipment?**

The kit is designed for use with the ONCORE Pro X system. Compatibility with other equipment is not guaranteed.

INTRODUCTION

This Setup Guide demonstrates the use of an HCR™ RNA-ISH Kit on the ONCORE Pro X platform from Biocare Medical. Reagent preparation steps, including registering individual ONCORE Pro vials for their respective reagents, will be described in further detail. Each ONCORE Pro X run takes approximately 10 hours followed by a short post-processing of stained slides. The HCR™ RNA-ISH Kit can be used to probe and visualize RNA transcripts in FFPE tissue sections. Please read through the Setup Guide for additional information so that you can easily incorporate the HCR™ RNA-ISH assay into your current workflow.

HCR™ RNA-ISH Kit Information

Upon receiving an HCR™ RNA-ISH Kit, please check all reagents and their storage conditions.

HCR™ RNA-ISH Starter Kit

HCR™ Reagents	Amount for an HCR™ RNA-ISH Starter Kit	Storage Temperature
HCR™ Probe A <i>PPIB/Ppib</i> ¹ – Positive Control ²	1 mL	2 to 8 °C
HCR™ Probe B <i>PPIB/Ppib</i> ¹ – Positive Control ²	1 mL	2 to 8 °C
HCR™ Probe A Diluent	3 mL	2 to 8 °C
HCR™ Probe B Diluent	3 mL	2 to 8 °C
HCR™ Probe A <i>dapB</i> – Negative Control	1 mL	2 to 8 °C
HCR™ Probe B <i>dapB</i> – Negative Control	1 mL	2 to 8 °C
HCR™ Probe A Diluent	3 mL	2 to 8 °C
HCR™ Probe B Diluent	3 mL	2 to 8 °C
HCR™ Membrane Stain ³	3 mL	2 to 8 °C
HCR™ Control Slides ⁴	3 Slides	2 to 8 °C
HCR™ Pretreat	5 mL	2 to 8 °C
HCR™ Detect A	5 mL	2 to 8 °C
HCR™ Detect B	7 mL	2 to 8 °C
HCR™ Detect C	5 mL	2 to 8 °C
HCR™ Detect D	7 mL	2 to 8 °C
HCR™ Detect E	7 mL	2 to 8 °C
HCR™ Detect F AP/HRP ⁵	7 mL	2 to 8 °C
HCR™ Post-Process A	5 mL	2 to 8 °C
HCR™ Post-Process B	7 mL	2 to 8 °C

1. Upper and lower cases are used to denote human and mouse HCR™ Probes respectively.
2. These are the HCR™ Probes included in the HCR™ RNA-ISH Starter Kit, and they are provided in volumes sufficient enough to perform the assay on 10 slides each.
3. The HCR™ Membrane Stain's host species is in rabbit and is provided in a volume sufficient to perform the assay on 5 slides.
4. The HCR™ Control Slides include 3 human or mouse liver FFPE tissue sections. Please allocate one slide for the positive control, one slide for the negative control, and one slide for the HCR™ RNA-ISH + IHC/IF co-detection assay using the HCR™ Membrane Stain.

5. HCR™ Detect F AP is included in the HCR™ RNA-ISH AP Starter Kit, and HCR™ Detect F HRP is included in the HCR™ RNA-ISH HRP Starter Kit.

HCR™ RNA-ISH Kit

HCR™ Reagents	Amount for a 20 Slide Kit	Amount for a 90 Slide Kit	Storage Temperature
HCR™ Probe A ¹	1.75 mL	7 mL	2 to 8 °C
HCR™ Probe B	1.75 mL	7 mL	2 to 8 °C
HCR™ Probe A Diluent	5.25 mL	21 mL	2 to 8 °C
HCR™ Probe B Diluent	5.25 mL	21 mL	2 to 8 °C
HCR™ Pretreat	5 mL	21 mL	2 to 8 °C
HCR™ Detect A	5 mL	21 mL	2 to 8 °C
HCR™ Detect B	7 mL	28 mL	2 to 8 °C
HCR™ Detect C	5 mL	21 mL	2 to 8 °C
HCR™ Detect D	7 mL	28 mL	2 to 8 °C
HCR™ Detect E	7 mL	28 mL	2 to 8 °C
HCR™ Detect F AP/HRP ²	7 mL	28 mL	2 to 8 °C
HCR™ Post-Process A	5 mL	21 mL	2 to 8 °C
HCR™ Post-Process B ³	7 mL	28 mL	2 to 8 °C

1. Every HCR™ Probe includes 4 components: HCR™ Probe A, HCR™ Probe B, HCR™ Probe A Diluent, and HCR™ Probe B Diluent. Please reference Page 13 for more information on how to prepare the HCR™ Probe Solution.
2. HCR™ Detect F AP is included in the HCR™ RNA-ISH AP Kit, and HCR™ Detect F HRP is included in the HCR™ RNA-ISH HRP Kit.
3. HCR™ Post-Process B is only needed if you are performing an HCR™ RNA-ISH + IHC/IF co-detection assay.

Required Materials for the ONCORE Pro X

The HCR™ RNA-ISH protocol requires specific materials available only from Biocare Medical. It is essential to check the availability of these materials prior to setting up an HCR™ RNA-ISH experiment. For more information, please inquire with your Biocare Medical representative.

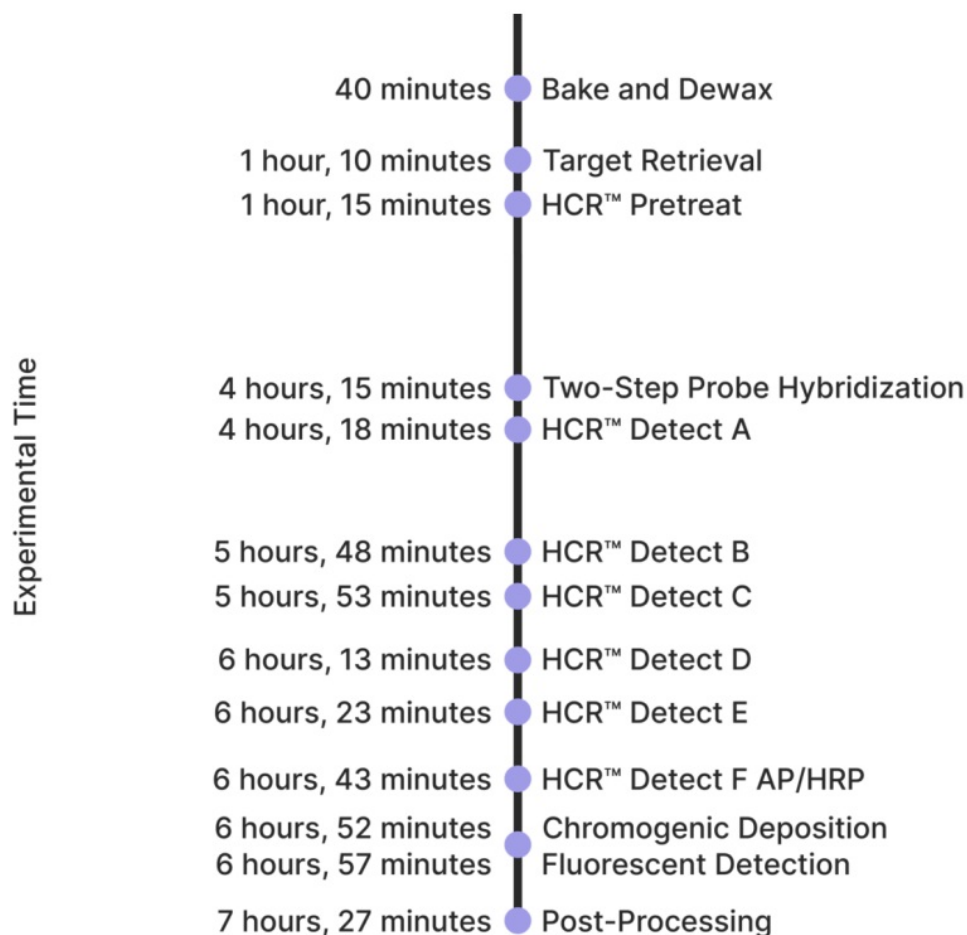
Materials from Biocare Medical		
	Catalog #	Storage Temperature
ONCORE Pro 7 mL Improv Reagent Vials	ONCPR101JJ	RT
ONCORE Pro 15 mL Improv Reagent Vials	ONCPR102JJ	RT
Dewax Solution 1, DS1 Kit	OPRI6001KT60	2 to 8 °C
ONCORE Pro Antigen Retrieval 1 (AR1), high pH	OPRI6006T60	2 to 8 °C
ONCORE Pro Hematoxylin	OPRI6087T60	2 to 8 °C
ONCORE Pro DAB Chromogen Kit ¹	OPRI6056KT180	2 to 8 °C
ONCORE Pro Warp Red Chromogen Kit ¹	OPRI6083KT60	2 to 8 °C
ONCORE Pro Rabbit HRP Detection ²	OPRI6008T60	2 to 8 °C
ONCORE Pro Rabbit AP Detection ²	OPRI6043T60	2 to 8 °C
ONCORE Pro Mouse HRP Detection ²	OPRI6007T60	2 to 8 °C
ONCORE Pro Mouse AP Detection ²	OPRI6044T60	2 to 8 °C

1. The HCR™ RNA-ISH protocol uses DAB and Warp Red chromogens for HRP and AP detection, respectively.
2. The Rabbit or Mouse Polymer Detection Kits are only needed for ISH + IHC/IF co-detection assays.

User-Supplied Materials

Materials from Other Vendors		
	Supplier	Comment
FFPE Sample Slides	Any	SuperFrost or SuperFrost® Plus slides are recommended for best results
Propar (xylene substitute)	Fisher Scientific	Xylene may be substituted
Drying Oven	Any	Capable of maintaining temperature at ~60 °C
BioCare EcoMount	Biocare Medical	Mounting medium compatible with all Biocare chromogens
Cytoseal	Any	Suitable mounting medium for HRP-driven chromogens
ProLong™ Gold Antifade Mountant with DAPI	Thermo Fisher	Mounting medium compatible with RNA-FISH and I F staining
Cover Glass	Any	Dimension depends on the size of the tissue
100% Ethanol	Any	None
Tyramide Dyes	Any	See Appendix E for Recommendations

Overall Workflow of the HCR™ RNA-ISH Protocol



As mentioned earlier, each ONCORE Pro X run takes approximately 10 hours. The timeline above only accounts for 7 hours and 27 minutes of this run (depending on whether you're performing the assay for chromogenic or fluorescent detection), as the remaining time comes from the additional ONCORE Pro X washing steps.

Set Up ONCORE Pro X Protocols

The four main steps in setting up an ONCORE Pro X protocol for an HCR™ RNA-ISH run are:

1. Register HCR™ RNA-ISH reagents in the ONCORE Pro X software
2. Create an HCR™ RNA-ISH protocol using an existing IHC staining protocol as a template
3. Select protocols for individual slide modules
4. Scan and map reagents and initiate the staining process

Register HCR™ RNA-ISH Reagents in the ONCORE Pro X Software

In order to create ONCORE Pro X staining protocols, reagent names associated with the HCR™ Reagents need to be registered in the ONCORE Pro X System Software. The table below lists all the reagents that need to be registered for the HCR™ RNA-ISH assay.

Oncore Pro Reagent Vials			
Name	Type of Container¹	Reagent Type	Viscosity Level
HCR™ Pretreat	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
HCR™ Probe A	7 mL or 15 mL ONCORE Pro Reagent Vial	CISH RNA	4
HCR™ Probe B	7 mL or 15 mL ONCORE Pro Reagent Vial	CISH RNA	4
HCR™ Detect A	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
HCR™ Detect B	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
HCR™ Detect C	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
HCR™ Detect D	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
HCR™ Detect E	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
HCR™ Detect F HRP/AP	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
HCR™ Post-Process A	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
HCR™ Post-Process B ²	7 mL or 15 mL ONCORE Pro Reagent Vial	Special	1
Primary Antibody ²	7 mL ONCORE Pro Reagent Vial	Special	1

We recommend using 7 mL and 15 mL Oncore Pro Reagent Vials for 20-slide and 90-slide kits, respectively. These reagents are only required for running an HCR™ RNA-ISH + IHC/IF co-detection assay.

1. To register HCR™ RNA-ISH reagents, open the ONCORE Pro X System Software and go to: System utilities → Reagent Editor → Select Reagent Type (e.g., CISH RNA) → Add New → Enter the HCR™ Reagent names → Check/change the viscosity level → Save

Reagent Editor

Reagent Types	Reagents
Buffer	CISH RNA Temp1
CISH DNA 1	DapB-RNA
CISH DNA 2	EBER Probe
CISH DNA 3	Glp1r-RNA dual E
CISH Extras	Glp1r-RNA dual O
CISH RNA	HCR Probe A
Cyto/HemeFISH	HCR Probe A V4
CytoFISH	HCR Probe B
Detection	HCR Probe B V4
Dewax	Hs PPIB-RNA
FISH Extras	Hs PPIB-RNA dual E
HemeFISH	Hs PPIB-RNA dual O
IHC Extras	Hs PPIB-RNA single E

Name: Hazardous: ☐ Yes

Type: Viscosity Level:

Protocol Name:

This reagent is:

2. Repeat Step 1 for all other HCR™ Reagents listed in the table above.

Create an HCR™ RNA-ISH Protocol Using an Existing IHC Staining Protocol as a Template

- The Advanced Protocol Editor is a software that enables you to create custom protocols with the help of a protocol template. After opening the Advanced Protocol Editor, select one of the pre-existing IHC protocols such as Ms HRP Temp1 and use it as a template.

Step	Set	Ext	Lnk	Reagent Type	Reagent	Vol(μL)	Total Inc Time	Temp(°C)	Heat Inc Time	Agitation	Wash Buffer	#	Vol (μL)
1	A	N	N	Dewax	DS1	240	00:05:00	65.00°	00:04:30	00:00:01 / 00:00:59 Thresh: 71.00 °C	System Fluid	0	85
2	A	Y	N	Dewax	DS2-50	200	00:04:30	58.00°	00:02:30	00:00:01 / 00:00:59 Thresh: 71.00 °C	Buffer	1	220
3	B	Y	N	Retrieval	AR1, high pH	370	00:32:00	101.00°	00:27:00	00:15:00 / 00:17:00 Thresh: 115.00 °C	System Fluid	1	200
4	B	Y	N	Buffer	System Fluid	220	00:04:20	33.00	00:59:00	00:00:01 / 00:00:59 Thresh: 71.00 °C	System Fluid	0	85
5	C	Y	N	Ms HRP	Ms HRP Temp1	130	00:30:00	RT	00:30:00	00:00:01 / 00:01:30 Thresh: 71.00 °C	System Fluid	1	240
6	D	Y	N	Buffer	System Fluid	220	00:00:10	RT	00:59:00	00:00:01 / 00:00:01 Thresh: 71.00 °C	System Fluid	0	200
7	D	Y	N	Detection	Mouse HRP	130	00:20:00	RT	00:59:00	00:00:01 / 00:01:30 Thresh: 71.00 °C	System Fluid	1	240
8	D	Y	N	Buffer	System Fluid	240	00:04:20	35.00	00:04:00	00:00:01 / 00:01:59 Thresh: 71.00 °C	System Fluid	0	85
9	D	Y	N	Buffer	System Fluid	240	00:04:20	35.00	00:04:00	00:00:01 / 00:01:59 Thresh: 71.00 °C	System Fluid	1	240

Ms HRP Template 1

Type

Primary Reagent

IHC Extras Temp1

Direct Rat-MBP-AP RT wash

HCR Secondary Chromogenic IHC Rat primary

HCR Secondary Chromogenic IHC Rat primary

Ms AP Temp1

HCR IHC

Ms HRP Temp1

IHC Frozen Temp1

Multiplex 1 Temp1

CISH DNA 3 Temp1

CISH RNA Temp1

PathoFISH Temp1

Cyto/HemeFISH Temp1

FISH Extras Temp1

Note: This template already includes the dewax and target retrieval steps used in the HCR™ RNA-ISH protocols. The target retrieval temperature should be set at 95 °C.

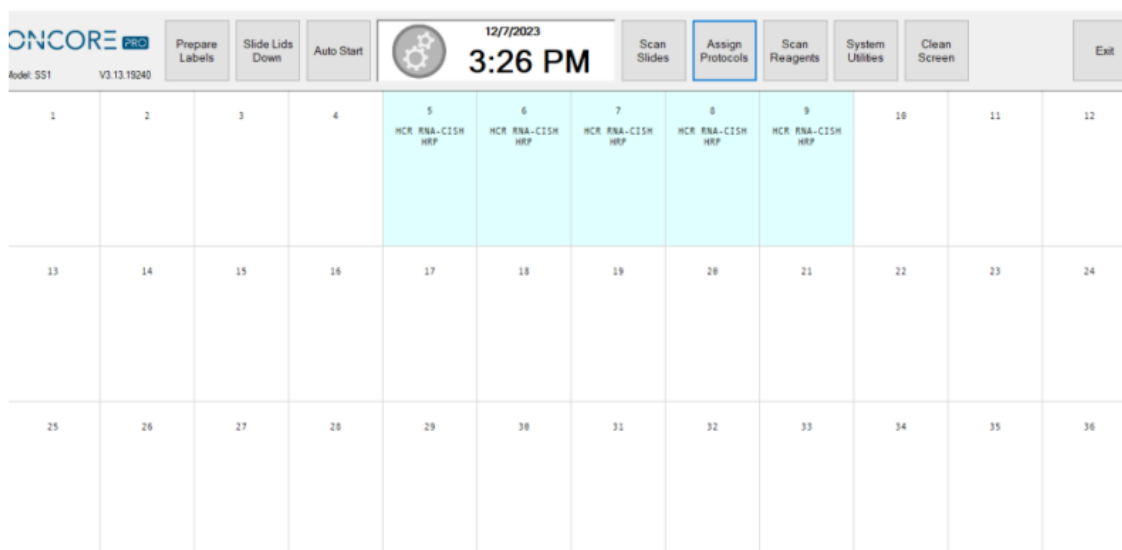
- There are three single-plex RNA-ISH protocols:
 - HCR™ RNA-CISH HRP (see pages 17-19)
 - HCR™ RNA-CISH AP (see pages 17-19)

3. HCR™ RNA-FISH (see pages 20-22)

	HCR™ RNA-CISH HRP	HCR™ RNA-CISH AP	HCR™ RNA-FISH
Main Differences:	Uses HCR™ Detect F HRP and an HRP-driven chromogen (e.g., DAB).	Uses HCR™ Detect F AP and an AP-driven chromogen (e.g., Warp Red).	Uses third-party Tyramide dyes. Hematoxylin and HCR™ Post-Process A should be omitted.

Note: Please see the Appendix for detailed staining protocols for each assay type (Appendices A and B) and third-party recommended Tyramide dyes (Appendix E).

- Slides could be labeled by either (1) preparing label options (see the ONCORE User Manual for more information) or (2) handwriting directly on the slides.
- Click on Slide Modules. This is where the slides will be stained after assigning protocols by clicking on Assign Protocols. Staining protocols created from the Advanced Protocol Editor will show up from the drop-down menu. Select the appropriate protocols (HCR™ RNA-CISH HRP is selected in the following figure).



Scan and Map Reagents and Initiate the Staining Process

Before initiating a run, all reagents used by the previously programmed protocols need to be either (1) scanned or (2) manually mapped. We recommend placing ready-to-use (RTU) Biocare reagents in the first two rows (i.e., positions 1-10), and the rest of the HCR™ Reagents in the other rows (i.e., positions 11-39).

- Click on Scan Reagents to allow the instrument to scan all the Biocare reagents. Once all the Biocare reagents have been scanned, click on Stop to conclude the scanning process. The unscanned HCR™ Reagents will appear in red.
- Map the HCR™ reagents to the proper positions by clicking on the Reagent Name First in the table. Then, click reagent position in the left panel. Following the manual assignment, the outcome should resemble the image provided below.

Reagent Name	Reagent Position	Number of Tests Required	Volume Required (µl x 100%)	Number of Tests Scanned	Expiration (YYYYMM)	Remarks
DS1	1	2	518	12	202403	BIOCARE MEDICAL, OPR6001T30
DS2-50	2	2	432	12	202403	BIOCARE MEDICAL, OPR6003T30
ARI1 high pH	3	2	800	12	202409	BIOCARE MEDICAL, OPR6006T30
HCR Probe A	19	2	476	2	202312	N/A, Manually Assigned
HCR Probe A V4	24	2	476	2	202312	N/A, Manually Assigned
HCR Probe B V4	25	2	476	2	202312	N/A, Manually Assigned
HCR Detect A	11	2	476	2	202312	N/A, Manually Assigned
HCR Detect B	12	2	476	2	202312	N/A, Manually Assigned
HCR Detect C	13	2	476	2	202312	N/A, Manually Assigned
HCR Detect D	14	2	476	2	202312	N/A, Manually Assigned
HCR Detect E	15	2	476	2	202312	N/A, Manually Assigned
HCR Detect F HRP	20	2	476	2	202312	N/A, Manually Assigned
DAB	40	2	432	2	202312	On Rack Mixing
DAB Buffer	5	4	813	20	202410	Reagent A for mixing DAB BIOCARE MEDICAL, OPR6055T60
DAB Chromogen	4	4	51	20	202410	Reagent B for mixing DAB BIOCARE MEDICAL, OPR6054T60
Hydrolytic	10	2	476	34	202410	BIOCARE MEDICAL, OPR6087T60
HCR Post Process A	18	2	476	2	202312	N/A, Manually Assigned

3. Initiate the HCR™ RNA-ISH run immediately by selecting Start Staining Process. Alternatively, Delay Start Staining Process allows you to schedule the run to finish at a specific time.

Post-Processing for HRP-Based Detection

After slides are unloaded from the ONCORE Pro X, we recommend washing the slides thoroughly with tap water. Dehydrate by immersing the slides in 95% ethanol for 3 minutes twice followed by 100% ethanol for 3 minutes twice. Then, immerse the slides in a xylene (or xylene substitute) solution for 5 minutes and mount one slide at a time with Cytoseal (or any other xylene-based mounting medium) or EcoMount. Allow slides to air dry for 5 minutes before imaging.

Post-Processing for AP-Based Detection

After slides are unloaded from the ONCORE Pro X, we recommend washing the slides thoroughly with tap water. Bake the slides for at least 15 minutes (or until dry) at 60 °C. Dip the slide in xylene (or xylene substitute) for 1-3 seconds and immediately apply EcoMount for cover-slipping.

Post-Processing for Fluorescent Detection

After slides are unloaded from the ONCORE Pro X, we recommend immersing the slides in 1 x PBST/TBST for 3 minutes. Mount one slide at a time with the ProLong™ Gold Antifade mounting medium (or any other suitable mounting medium that you are familiar with).

Reagent Preparation

The three main steps to prepare for an HCR™ RNA-ISH run are:

1. Prepare Probe Solutions
2. Estimate the amount of probe solutions needed (this is an optional step)
3. Transfer all HCR™ Reagents to the ONCORE Vials (7 mL or 15 mL)

Preparation of Probe Solutions

HCR™ Probe Hybridization is a two-step process that requires two separate probe solutions. HCR™ Probe A Solution and HCR™ Probe B Solution can be prepared separately by mixing HCR™ Probe A with HCR™ Probe A Diluent and HCR™ Probe B with HCR™ Probe B Diluent, respectively. We recommend transferring the entirety of HCR™ Probe A and HCR™ Probe B into the HCR™ Probe A Diluent and HCR™ Probe B Diluent bottles. After the solutions have been transferred, you can either vortex the bottles or invert the bottle 4-5 times to ensure proper mixture.

Estimation of Required Probe Solutions

- To calculate the exact quantity of probe solution needed, it is important to consider the dead volume of an ONCORE Vial. The volumes needed for N number of slides can be calculated using the formula for the ONCORE Pro X listed below:
 - $220 \times 1.08 \times N + 500 \text{ } \mu\text{L}$ (dead volume) for a 7 mL ONCORE Vial
 - $220 \times 1.08 \times N + 1000 \text{ } \mu\text{L}$ (dead volume) for a 15 mL ONCORE Vial
- The amount of each probe solution needed per slide is 220 μL . The table below shows the volume of probe solution needed for staining 3 slides while taking the dead volumes into account.

Container Type	Probe Solution Volume	Amount of HCR™ Reagent
7 mL ONCORE Vial	1213 μL ($220 \times 1.08 \times 3 + 500$)	Transfer 1213 μL of probe solution to the registered probe vials (e.g., PPIB HCR™ Probe A and PPIB HCR™ Probe B)
15 mL ONCORE Vial	1713 μL ($220 \times 1.08 \times 3 + 1000$)	Transfer 1713 μL of probe solution to the registered probe vials (e.g., PPIB HCR™ Probe A and PPIB HCR™ Probe B)

NOTE: You can choose to transfer all probe solutions (~7 mL for each probe) to the respective ONCORE Pro Vials. Store the Vials at 4 °C after each run.

Reagent Transfer and Storage

Instructions for reagent transfer can be found in the table below. Please transfer all HCR™ RNA-ISH reagents to their name-associated vials. Store all ONCORE Pro X Vials in 4C fridge after use.

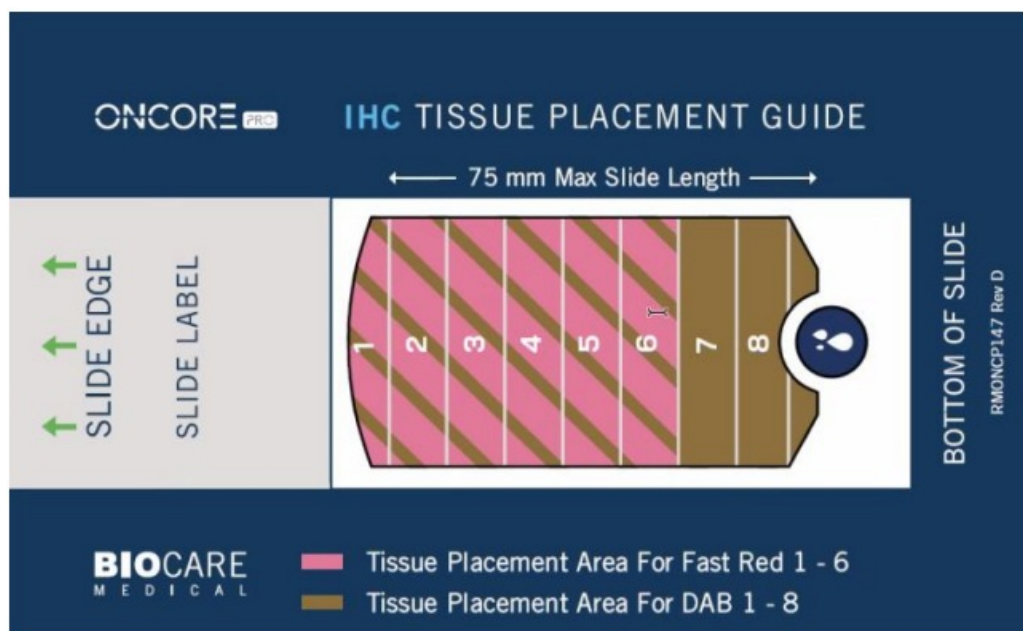
Oncore Vials	Instructions ¹	Storage Conditions
HCR™ Pretreat	Transfer the entirety of HCR™ Pretreat to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Probe A Solution	Transfer the entirety of or the calculated amount of HCR™ Probe A Solution to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Probe B Solution	Transfer the entirety of or the calculated amount of HCR™ Probe B Solution to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Detect A	Transfer entirety of HCR™ Detect A to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Detect B	Transfer entirety of HCR™ Detect B to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Detect C	Transfer entirety of HCR™ Detect C to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Detect D	Transfer entirety of HCR™ Detect D to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Detect E	Transfer entirety of HCR™ Detect E to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Detect F AP	Transfer entirety of HCR™ Detect F AP to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Detect F HRP	Transfer entirety of HCR™ Detect F HRP to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Post-Process A	Transfer entirety of HCR™ Post-Process A to the 7 mL or 15 mL Vial	Store at 4 °C after use
HCR™ Post-Process B	Transfer entirety of HCR™ Post-Process B to the 7 mL or 15 mL Vial	Store at 4 °C after use
Primary Antibody	Transfer entirety of HCR™ Membrane Stain ² to the 7 mL Vial	Store at 4 °C after use

- Note:** Use 7 mL and 15 mL vials for 20-slide and 90-slide kits respectively. The HCR™ Reagent volumes for 90-slide kits will be more than 15 mL. You can refill the 15 mL vials as needed after each run.
- The HCR™ Membrane Stain is only provided in the HCR™ RNA-ISH Starter Kit.

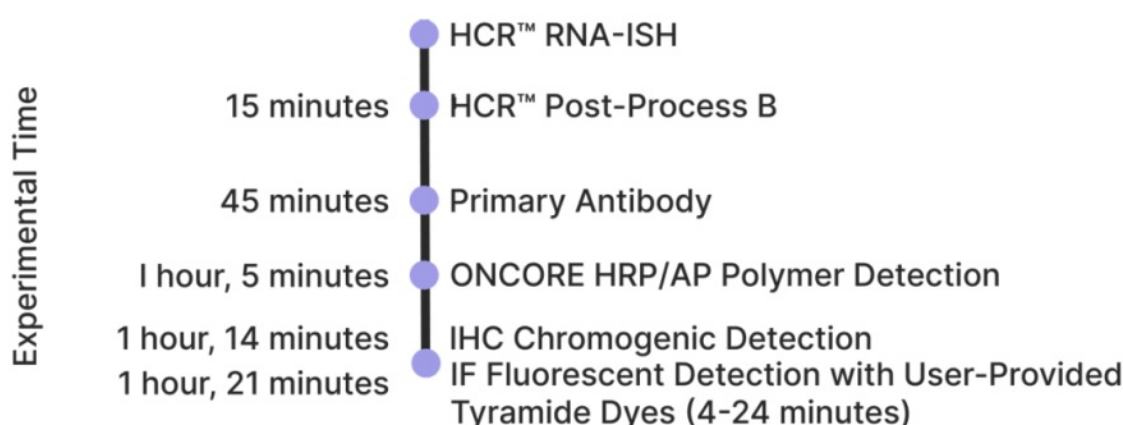
Other important notes:

- Please avoid excessive bubbles when transferring HCR™ Probe and HCR™ Detect Solutions to the 7 mL or 15 mL ONCORE Vials.
- If the reagent-filled ONCORE Vials have not been used for more than a week, it is highly recommended to gently invert the vials several times with the lid closed to ensure solution uniformity before next use.
- Please note that the use of Warp Red and Fast Red Biocare chromogens can sometimes result in non-uniform staining for tissues placed close to the injection port. To mitigate the risk of non-uniform staining, Biocare

suggests placing your tissue samples in a designated area, as illustrated in the image below.



Overall Workflow of the HCR™ RNA-ISH + IHC/IF Protocol



Creating an HCR™ RNA-ISH + IHC/IF Co-Detection Protocol

The additional reagents needed to run an HCR™ RNA-ISH + IHC/IF co-detection assay are:

1. HCR™ Post-Process B
2. User-provided primary antibody (or the HCR™ Membrane Stain from the HCR™ RNA-ISH Starter Kit)
3. Anti-Ms, Rb AP-, or HRP-conjugated polymer detection kits from Biocare Medical
4. HRP- or AP-driven chromogens for IHC or third-party Tyramide-conjugated fluorophores for IF

Create an HCR™ RNA-CISH + IHC Co-Detection Protocol

This co-detection protocol can be created using the previously programmed HCR™ RNA-CISH HRP/AP protocol as a template. Please follow the detailed HCR™ RNA-CISH + IHC co-detection protocol (see pages 23 to 26) in Appendix C for additional steps.

Note: HCR™ Post-Process B (steps 33 to 34) is not needed if HCR™ RNA-CISH HRP detection is followed by AP-driven IHC detection.

Create an HCR™ RNA-FISH + IF Co-Detection Protocol

This co-detection protocol can be created using the previously programmed HCR™ RNA-FISH protocol as a

template. Please follow the detailed HCR™ RNA-FISH + IF co-detection protocol (see pages 27-29) in Appendix D for additional steps.

Note: You will need to provide another Tyramide dye for IF that is spectrally different from the Tyramide dye used for HCR™ RNA-FISH. See Appendix E for a list of recommended third-party Tyramide dyes and their suggested starting concentrations.

Appendix

Appendix A: Detailed Staining Protocol for HCR™ RNA-CISH [Chromogenic Detection]

Step No.	Reagent Type	Reagent name	Reagent Vol. (μL)	Total Inc. Time	Temp (°C)	Heat Inc. Time	Agitation	#	Wash Buffer Vol. (μL)
1	Dewax	DS1 (No extraction)	240	00:05:00	65.00	00:04:30	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	0	85
2	Dewax	DS2-50	200	00:04:30	58.00	00:02:30	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	1	220
3	Retrieval	AR1, high pH	370	00:32:00	95.00	00:27:00	Lid up: 15 min (A3) Lid down: 17 min (A4)	1	200
4	Buffer	System Fluid	220	00:04:20	33.00	00:59:00	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	1	85
5	Buffer	System Fluid	220	00:00:10	RT	00:00:00	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	220

6	Special	HCR™ Pretreat	220	00:05:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	1	240
7	Buffer	System Fluid	220	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	200
8	CISH RNA	HCR™ Probe A	220	01:30:00	45.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
9	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
10	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
11	Buffer	System Fluid	240	00:05:00	45.00	00:05:00	Lid up: 1 sec (injection) Lid down: 85 sec (A4)	0	85
12	CISH RNA	HCR™ Probe B	220	01:30:00	45.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240

13	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
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14	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
15	Buffer	System Fluid	240	00:05:00	45.00	00:05:00	Lid up: 1 sec (injection) Lid down: 85 sec (A4)	0	85
16	Special	HCR™ Detect A	220	00:03:00	42.00	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	0	240
17	Special	HCR™ Detect B	220	01:30:00	42.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
18	Buffer	System Fluid	240	00:01:00	42.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
19	Buffer	System Fluid	240	00:05:00	42.00	00:05:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240

20	Special	HCR™ Detect C	220	00:05:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	0	85
21	Special	HCR™ Detect D	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
22	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	85
23	Buffer	System Fluid	240	00:03:00	RT	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	0	85
24	Special	HCR™ Detect E	220	00:12:00	RT	00:12:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
25	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
26	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85

27*	Special	HCR™ Detect F HRP*	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
28	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

29	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
30*	Detection	DAB*	220	00:08:40	37.00	00:08:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240
31	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	
32	Others	Hematoxylin	220	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 30 sec (A4)	2	240
33	Buffer	System Fluid	220	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	85
34	Special	HCR™ Post-Process A	220	00:03:00	RT	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	1	240
35	Buffer	System Fluid	130	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (Down)	0	85

* For AP-based detection, please make changes to steps 27 and 30 as follows:

27*	Special	HCR™ Detect F AP*	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
30*	Detection	Warp Red*	220	00:07:00	RT	00:09:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240

Appendix B: Detailed Staining Protocol for HCR™ RNA-FISH [Fluorescent Detection]

Step No.	Reagent Type	Reagent name	Reagent Vol. (μL)	Total Inc. Time	Temp (°C)	Heat Inc. Time	Agitation	#	Wash Buffer Vol. (μL)
1	Dewax	DS1 (No extraction)	240	00:05:00	65.00	00:04:30	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	0	85
2	Dewax	DS2-50	200	00:04:30	58.00	00:02:30	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	1	220
3	Retrieval	AR1, high pH	370	00:32:00	95.00	00:27:00	Lid up: 15 min (A3) Lid down: 17 min (A4)	1	200
4	Buffer	System Fluid	220	00:04:20	33.00	00:59:00	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	1	85

5	Buffer	System Fluid	220	00:00:10	RT	00:00:00	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	220
6	Special	HCR™ Pretreat	220	00:05:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	1	240
7	Buffer	System Fluid	220	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	200
8	CISH RN A*	HCR™ Probe A	220	01:30:00	45.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
9	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
10	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
11	Buffer	System Fluid	240	00:05:00	45.00	00:05:00	Lid up: 1 sec (injection) Lid down: 85 sec (A4)	0	85

12	CISH RN A*	HCR™ Probe B	220	01:30:00	45.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
13	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
14	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

15	Buffer	System Fluid	240	00:05:00	45.00	00:05:00	Lid up: 1 sec (injection) Lid down: 85 sec (A4)	0	85
16	Special	HCR™ Detect A	220	00:03:00	42.00	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	0	240
17	Special	HCR™ Detect B	220	01:30:00	42.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
18	Buffer	System Fluid	240	00:01:00	42.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

19	Buffer	System Fluid	240	00:05:00	42.00	00:05:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240
20	Special	HCR™ Detect C	220	00:05:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	0	85
21	Special	HCR™ Detect D	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
22	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	85
23	Buffer	System Fluid	240	00:03:00	RT	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	0	85
24	Special	HCR™ Detect E	220	00:12:00	RT	00:12:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
25	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

26	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85
27	Special	HCR™ Detect F HRP	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
28	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
29	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

30*	Special	Tyramide Dye 1**	220	00:15:00 [#]	RT	00:17:00	Lid up: 1 sec (injection) Lid down: 2 min 30 sec (A4)	2	240
31	Buffer	System Fluid	240	00:01:00	RT	00:02:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	240
32	Buffer	System Fluid	130	00:01:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 15 sec (Down)	0	85

- Please keep in mind that while CISH RNA is used here as the reagent type, the HCR™ Probes will still work as expected for fluorescent detection.

- The incubation time of the Tyramide dye listed here is only used for reference and you will need to optimize the actual incubation time

Appendix C: Detailed Staining Protocol for HCR™ RNA-CISH + IHC Co-Detection

Step No.	Reagent Type	Reagent name	Reagent Vol. (μL)	Total Inc. Time	Temp (°C)	Heat Inc. Time	Agitation	#	Wash Buffer Vol. (μL)
1	Dewax	DS1 (No extraction)	240	00:05:00	65.00	00:04:30	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	0	85
2	Dewax	DS2-50	200	00:04:30	58.00	00:02:30	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	1	220
3	Retrieval	AR1, high pH	370	00:32:00	95.00	00:27:00	Lid up: 15 min (A3) Lid down: 17 min (A4)	1	200
4	Buffer	System Fluid	220	00:04:20	33.00	00:59:00	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	1	85
5	Buffer	System Fluid	220	00:00:10	RT	00:00:00	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	220
6	Special	HCR™ Pretreat	220	00:05:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	1	240

7	Buffer	System Fluid	220	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	200
8	CISH RNA	HCR™ Probe A	220	01:30:00	45.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
9	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
10	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
11	Buffer	System Fluid	240	00:05:00	45.00	00:05:00	Lid up: 1 sec (injection) Lid down: 85 sec (A4)	0	85
12	CISH RNA	HCR™ Probe B	220	01:30:00	45.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
13	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
14	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

15	Buffer	System Fluid	240	00:05:00	45.00	00:05:00	Lid up: 1 sec (injection) Lid down: 85 sec (A4)	0	85
16	Special	HCR™ Detect A	220	00:03:00	42.00	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	0	240
17	Special	HCR™ Detect B	220	01:30:00	42.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
18	Buffer	System Fluid	240	00:01:00	42.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
19	Buffer	System Fluid	240	00:05:00	42.00	00:05:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240
20	Special	HCR™ Detect C	220	00:05:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	0	85
21	Special	HCR™ Detect D	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240

22	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	85
23	Buffer	System Fluid	240	00:03:00	RT	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	0	85
24	Special	HCR™ Detect E	220	00:12:00	RT	00:12:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
25	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
26	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85
27	Special	HCR™ Detect F AP	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
28	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

29	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
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30	Detection	Warp Red	220	00:07:00	RT	00:17:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	2	240
31	Buffer	System Fluid	130	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A7)	0	85
32	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85
33	Special	HCR™ Post-Process B	220	00:15:00	42.00	00:15:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240
34	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
35	Special	Primary Antibody	130	00:30:00	RT	00:30:00	Lid up: 1 sec (injection) Lid down: 2 min 30 sec (A7)	1	240

36	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85
37	Detection	Rabbit HRP*	130	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 90 sec (A7)	1	240
38	Buffer	System Fluid	240	00:04:20	35	00:04:00	Lid up: 1 sec (injection) Lid down: 2 min (A4)	0	85
39	Buffer	System Fluid	240	00:04:20	35	00:04:00	Lid up: 1 sec (injection) Lid down: 2 min (A4)	1	240
40	Detection	DAB**	220	00:08:40	37.00	00:08:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240
41	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	
42	Others	Hematoxylin	220	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 30 sec (A4)	2	240

43	Buffer	System Fluid	220	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	85
44	Special	HCR™ Post-Process A	220	00:03:00	RT	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	1	240

45	Buffer	System Fluid	130	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (Down)	0	85
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- Choose the appropriate ONCORE Polymer Detection based on the species (Rabbit or Mouse) of the primary antibody and the type of chromogen (HRP- or AP-driven) used.
- DAB is used here as an example. You may use other chromogens instead.

Appendix D: Detailed Staining Protocol for HCR™ RNA-FISH + IF Co-Detection

Step No.	Reagent Type	Reagent name	Reagent Vol. (μL)	Total Inc. Time	Temp (°C)	Heat Inc. Time	Agitation	#	Wash Buffer Vol. (μL)
1	Dewax	DS1 (No extraction)	240	00:05:00	65.00	00:04:30	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	0	85
2	Dewax	DS2-50	200	00:04:30	58.00	00:02:30	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	1	220

3	Retrieval	AR1, high pH	370	00:32:00	95.00	00:27:00	Lid up: 15 min (A3) Lid down: 17 min (A4)	1	200
4	Buffer	System Fluid	220	00:04:20	33.00	00:59:00	Lid up: 1 sec (injection) Lid down: 59 sec (A4)	1	85
5	Buffer	System Fluid	220	00:00:10	RT	00:00:00	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	220
6	Special	HCR Pretreat	220	00:05:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	1	240
7	Buffer	System Fluid	220	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	200
8	CISH RNA*	HCR™ Probe A	220	01:30:00	45.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
9	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

10	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
11	Buffer	System Fluid	240	00:05:00	45.00	00:05:00	Lid up: 1 sec (injection) Lid down: 85 sec (A4)	0	85
12	CISH RNA*	HCR™ Probe B	220	01:30:00	45.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
13	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
14	Buffer	System Fluid	240	00:01:00	45.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

15	Buffer	System Fluid	240	00:05:00	45.00	00:05:00	Lid up: 1 sec (injection) Lid down: 85 sec (A4)	0	85
16	Special	HCR™ Detect A	220	00:03:00	42.00	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	0	240

17	Special	HCR™ Detect B	220	01:30:00	42.00	01:30:00	Lid up: 1 sec (injection) Lid down: 30 min (A4)	1	240
18	Buffer	System Fluid	240	00:01:00	42.00	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
19	Buffer	System Fluid	240	00:05:00	42.00	00:05:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240
20	Special	HCR™ Detect C	220	00:05:00	RT	00:05:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	0	85
21	Special	HCR™ Detect D	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
22	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 15 sec (A4)	0	85
23	Buffer	System Fluid	240	00:03:00	RT	00:03:00	Lid up: 1 sec (injection) Lid down: 1 min (A4)	0	85

24	Special	HCR™ Detect E	220	00:12:00	RT	00:12:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
25	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
26	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85
27	Special	HCR™ Detect F HRP	220	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 3 min (A4)	1	240
28	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
29	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85

30	Special	Tyramide Dye 1	220	00:15:00	RT	00:17:00	Lid up: 1 sec (injection) Lid down: 2 min 30 sec (A4)	2	240
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31	Buffer	System Fluid	130	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A7)	0	85
32	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85
33	Special	HCR™ Post-Process B	220	00:15:00	42.00	00:15:00	Lid up: 1 sec (injection) Lid down: 90 sec (A4)	1	240
34	Buffer	System Fluid	240	00:01:00	RT	00:01:00	Lid up: 1 sec (injection) Lid down: 25 sec (A4)	0	85
35	Special	Primary Antibody	130	00:30:00	RT	00:30:00	Lid up: 1 sec (injection) Lid down: 2 min 30 sec (A7)	1	240
36	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85
37	Detection	Rabbit HRP	130	00:20:00	RT	00:20:00	Lid up: 1 sec (injection) Lid down: 90 sec (A7)	1	240

38	Buffer	System Fluid	240	00:04:20	35	00:04:00	Lid up: 1 sec (injection) Lid down: 2 min (A4)	0	85
39	Buffer	System Fluid	240	00:04:20	35	00:04:00	Lid up: 1 sec (injection) Lid down: 2 min (A4)	1	240
40	Special	Tyramide Dye 2	220	00:15:00	RT	00:17:00	Lid up: 1 sec (injection) Lid down: 2 min 30 sec (A4)	2	240
41	Buffer	System Fluid	240	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (A4)	0	85
42	Buffer	System Fluid	130	00:00:10	RT	00:00:10	Lid up: 1 sec (injection) Lid down: 1 sec (Down)	0	85

Appendix E: Third-Party Recommended Tyramide Dyes

Validated Tyramide Dyes	Incubation Time	Recommended Starting Concentration	Vendor	Catalog #
CF488A	8-24 min	5 μ M	Biotium	92171
CF550R	8-24 min	5 μ M	Biotium	96077
CF555	8-24 min	5 μ M	Biotium	96021
CF583R	8-24 min	5 μ M	Biotium	96085
CF594	8-24 min	5 μ M	Biotium	92174
CF640R	8-24 min	5 μ M	Biotium	92175
CF754	8-24 min	5 μ M	Biotium	96090
Alexa Fluor 488 – Tyramide	8-24 min	1x	ThermoFisher	B40953
Alexa Fluor 546 – Tyramide	8-24 min	1x	ThermoFisher	B40954
Alexa Fluor 647 – Tyramide	8-24 min	1x	ThermoFisher	B40958
Alexa Fluor 750 – Tyramide	8-24 min	1x	ThermoFisher	B56131
Opal 520	8-24 min	1:500	Akoya Biosciences	FP1487001KT
Opal 570	8-24 min	1:500	Akoya Biosciences	FP1488001KT
Opal 620	8-24 min	1:500	Akoya Biosciences	FP1495001KT
Opal 690	8-24 min	1:500	Akoya Biosciences	FP1497001KT

Documents / Resources

	HCR RNA-ISH Setup Oncore Kit [pdf] Instructions RNA-ISH Setup Oncore Kit, Setup Oncore Kit, Oncore Kit, Kit
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References

- [B ONCORE Pro Improv Reagent Vials - Biocare Medical](#)
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- **T** [Tyramide Conjugates](#)
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