Get to Know HCR™ RNA-ISH by Molecular Instruments!

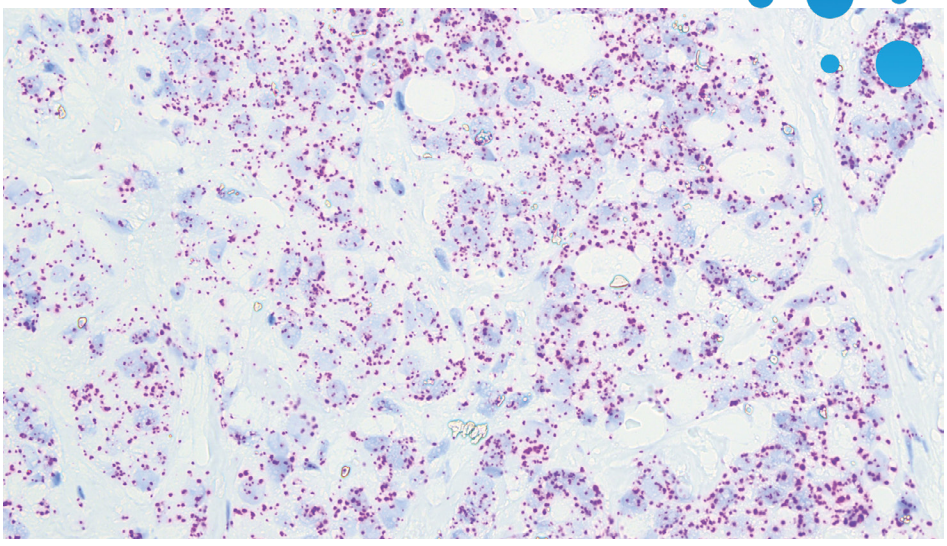
Molecular Instruments® (MI) designs and manufactures kits for automated and manual in situ hybridization (ISH) assays, offering no-charge custom probe design and compatibility with both brightfield and fluorescent microscopy. Researchers can take advantage of the next-generation HCR™ Platform, featuring unparalleled performance, speed, and versatility. HCR™ RNA-ISH is the only assay available that has been optimized without requiring destructive enzyme/protease digestion, enabling tissue morphology preservation, native compatibility with existing IHC/IF assays, and straightforward image analysis.

MI develops and synthesizes kits powered by its innovative HCR™ Platform for bioimaging applications in academic research, drug development, and clinical pathology and diagnostics. The HCR™ Platform introduced the concept of conditional nucleic acid self-assembly – a foundational principle for the field of dynamic nucleic acid nanotechnology.

Automation on the DISCOVERY ULTRA Platform

Get to know HCR™ RNA-ISH by Molecular Instruments!

- **Achieve greater consistency** from slide-to-slide, day-to-day and site-to-site with automated protocols.
- **Free up lab personnel** - Do more valuable work, while reducing staining failure due to human error.
- **Run different protocols simultaneously** - Different techniques such as fluorescent or chromogenic IHC, FISH/CISH, and IHC & ISH on the same slide, can be run simultaneously.



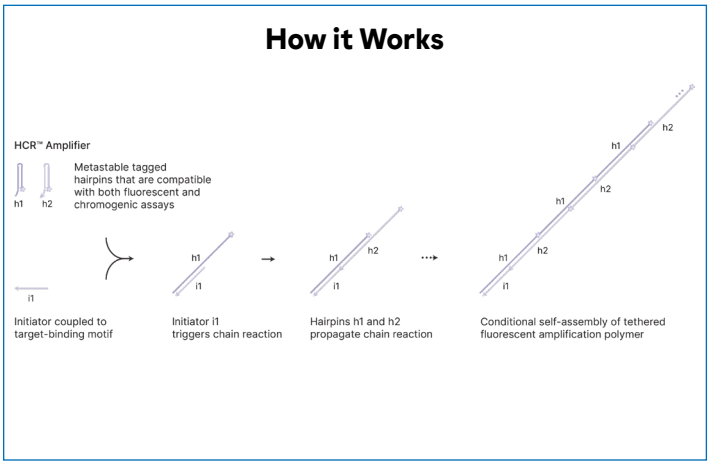
HCR™ RNA-CISH using a probe to mRNA from the glucagon-like peptide 1 receptor gene (GLP1R) in FFPE murine duodenum. 20X magnification.

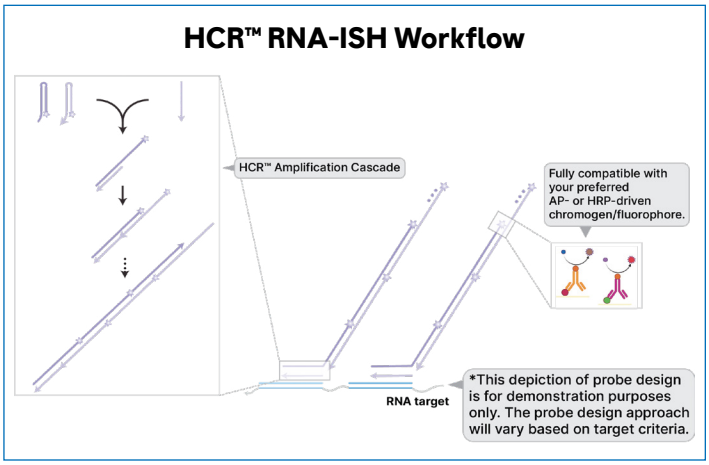
How does the HCR™ RNA-ISH Platform Work?

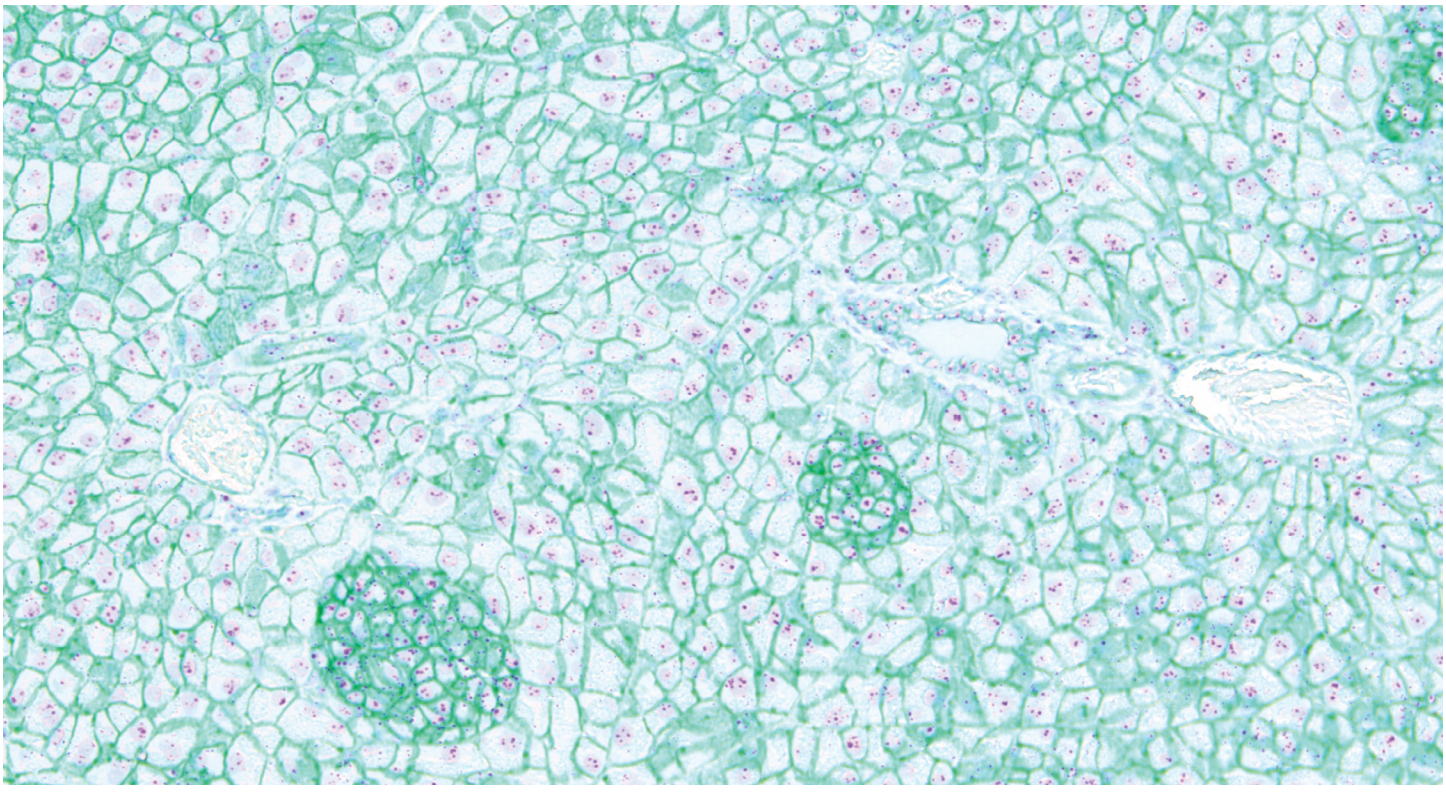
HCR™ Amplifiers are engineered oligonucleotides that in their normal state are folded into “hairpin” shapes as depicted in the following graphic. The cascading self-assembly that results from the reaction between the initiator and the hairpins creates a polymer of reporters attached to each target thereby enabling signal amplification. The HCR™ Platform is completely enzyme-free, isothermal at room temperature, and compatible with both fluorescent or brightfield microscopy.

Target any RNA sequence in any FFPE tissue with unparalleled performance, speed, and versatility.

This assay has been optimized without requiring destructive protease digestion, enabling tissue morphology preservation, native compatibility with existing IHC workflows, and straightforward image analysis. Molecular Instruments leads the industry by offering complimentary custom probe design and its catalog of tens of thousands of ready-to-ship HCR™ Probes. Flexible probe design can also address short targets with a three week turnaround time for custom HCR™ Probes.







Combined ISH and IHC on the same slide: HCR™ RNA-ISH using a probe to detect mRNA from the RNA polymerase II subunit A gene (Polr2a) and mRNA Purple-HRP and immunohistochemistry (IHC) to detect the sodium potassium ATPase (NaKATPase) protein with DISCOVERY Green-HRP. 10x magnification.

What are the Capabilities of the HCR™ Platform on the Ventana DISCOVERY ULTRA?

Protease-free sample preparation
Preserves tissue morphology and enables simultaneous protein detection

Native Compatibility for IHC
Easily integrate with existing IHC assays

Best-in-class RNA-ISH
Enables ISH in a wide variety of sample types

Compatible with a variety of different chromogens and fluorophores

Automation friendly
HCR™ RNA-ISH fully supports automation with Setup Guides and Starter Kits available for the Roche DISCOVERY ULTRA.

Straightforward pricing
Each HCR™ RNA-ISH Kit comes with all the necessary reagents needed (up to the chromogen/fluorophore) that are made ready-to use for any target of interest

Ventana DISCOVERY Chromogens Offer Key Advantages

The Research and Development Team at Ventana have developed several new chromogens to expand ISH and IHC multiplexing capabilities in brightfield microscopy. These new chromogens are based on fluorophores to allow for unique color generation and very narrow-range light absorption to improve compatibility for ISH and IHC multiplexing. By basing IHC/ISH chromogen chemistry on fluorophores, Ventana researchers have generated new, narrow-absorbance colors such as DISCOVERY HRP-driven Purple, Red, Yellow, Blue, Green and Teal. Tyramide chemistry is used to covalently deposit these unique chromogens for excellent stability.

- **Greater Control** over staining intensity than traditional chromogens
- **Permanently Bound** HRP-driven tyramide deposition
- **Distinct, strong** high-quality staining
- **Increases the Flexibility** of multiplexing IHC and ISH
- **Greater Stability** in EtOH and xylene than traditional chromogens
- **No Mixing!** Ready-to-Use chromogens

Ready-to-Use Fluorescent Options for HCR ™ RNA-FISH

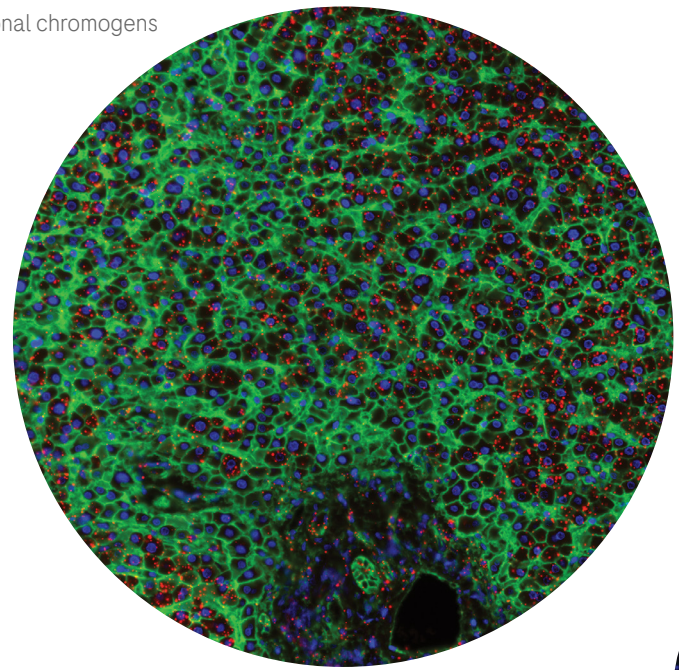
- **Bright, Stable Signal** covalently deposited by tyramide chemistry
- **No Mixing!** Ready-to-Use dispensers
- **More Choices** seven fluorescent options
- **Widely Compatible** standard emission spectra works with most imaging systems

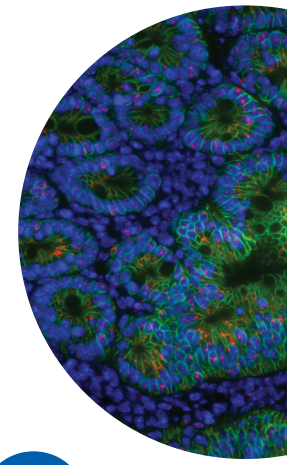
Translucent chromogens enable multiplexed IHC co-localization.

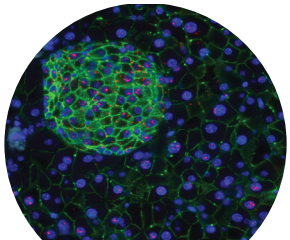
If the biomarkers of interest are present in the same sub-cellular compartment (nucleus, cytoplasm or cell membrane) this overlapping expression of targets is a key consideration when selecting chromogen options for IHC multiplexing. When designing a multiplexed chromogenic IHC assay that includes overlapping target biomarker expression, opaque colors such as DAB and Silver should not be used.

Traditionally, analysis of overlapping targets has been accomplished using immunofluorescence, but the availability of new Ventana translucent chromogens has provided an alternative method - in brightfield! Translucent chromogens allow a color shift when both colors are present in the same cell and sub-cellular compartment.

Availability of translucent chromogens such as Purple, Yellow, and Teal has opened up the ability to visualize overlapping targets in brightfield IHC or ISH multiplexed assays.







Creating your first HCR™ RNA-ISH order is simple

Detect any RNA target with no custom design fees in three easy steps:

1.  **Select an organism**
2.  **Pick any RNA target**
3.  **Specify your enzyme (AP or HRP)**

Ordering Information

CLICK TO ORDER

MOLECULAR INSTRUMENTS

Product	Quantity	Order Number
HCR™ RNA-ISH Starter Kit Designed to be an accessible introductory kit for evaluation of HCR™ RNA-ISH. Contains all reagents for the detection of one positive control (PPIB for human, Ppib for mouse), one negative control (dapB), one HCR™ Membrane Stain for co-detection, and HCR™ Control Slides for FFPE human/mouse liver.	1 kit (for 20 slides)	Varies
HCR™ Probe Kit Contains an HCR™ Probe against any target of interest.	1 kit (for 20 or 90 slides)	Varies
HCR™ Detect Kit Contains all necessary reagents for HCR™ RNA-ISH Amplification.	1 kit (for 20 or 90 slides)	Varies

ROCHE DIAGNOSTICS

Materials	Order Number
3 PRETREATMENT User-fillable Dispensers	Choose unique numbers for each
2 PROBE User-fillable Dispensers	
DETECTION User-fillable Dispensers	
DISCOVERY Teal HRP Detection Kit	08352941001
DISCOVERY mRNA Green HRP Kit	08952612001
DISCOVERY mRNA DAB Detection Kit	06614353001
DISCOVERY Purple HRP detection Kit	08352909001
DISCOVERY Alk Phos Red Kit	07425333001

= Dispensers for reagents from Molecular Instruments

= Chromogens

Materials	Order Number
Hematoxylin II	05277965001
Bluing Reagent	05266769001
Discovery Inhibitor RUO	07017944001
DISCOVERY Rhodamine 6G Kit	07988168001
DISCOVERY Cy5 Kit	07551215001
DISCOVERY FAM KIT	07988150001
DISCOVERY Red 610 FAM Kit	07988176001
DISCOVERY DCC Kit	07988192001

= Fluorescent Detection

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