HCR™ Gold IF Quick Reference Guide

This Quick Reference Guide introduces the breakthrough HCR™ HiFi Encoder for robustly encoding best-in-class quantitative signal amplification to your trusted 1° antibodies for game-changing same-species/isotype multiplexing using HCR™ Gold IF.

Technical support: support@molecularinstruments.com

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HCR™ HiFi Encoder Highlights

The HCR™ HiFi Encoder enables you to encode your favorite 1° antibodies for use with HCR™ Gold Amplifiers. At launch, encoders will be available for use with two host species/isotypes of primary antibodies (mouse IgG1 and rabbit IgG) and three HCR™ Gold Amplifiers (X1, X2, X3). Coming soon, additional encoders will be released to support more primary host species/isotypes and all ten HCR™ Gold Amplifiers (X1-X10).

Key Properties

- **Robust Amplification Encoding:** Robustly encode your favorite primary antibodies for use with HCR™ Gold Amplifiers without affecting target-binding affinity.
- **Same-Species/Isotype Multiplexing:** Perform multiplex IF using multiple 1° antibodies from the same host species/isotype with no cross-reactivity. For example, perform N-plex HCR™ Gold IF using rabbit IgG primary antibodies for all N target proteins at the same time.
- **Best-In-Class Amplification:** Exploit 1-step quantitative HCR™ Gold signal amplification for protein imaging, leveraging the dynamic nanotechnology that has redefined the state-of-the-art for RNA imaging.
- **Compatibility with HCR™ Gold RNA-FISH:** Perform unified multiplex, quantitative, high-resolution RNA and protein imaging by combining HCR™ Gold RNA-FISH with HCR™ Gold IF.

HCR™ Gold IF Workflow

Sample Preparation

Samples should be prepared in the same manner as for traditional immunofluorescence assays.

Encoding

The HCR™ HiFi Encoder encodes unmodified primary antibodies (mouse IgG1 or rabbit IgG) with the ability to trigger HCR™ Gold amplification (X1, X2, or X3). To encode, separately prepare each primary antibody at its standard working dilution; the encoding process for each antibody is completed at room temperature in ten minutes. The resulting HCR™ HiFi Antibody Probe solution is ready for use in the HCR™ Gold IF assay.

Antibody Binding

HCR™ HiFi Antibody Probe solution is applied directly to the sample. Incubate using the same conditions optimized for traditional immunofluorescence assays. For new assays, you may need to optimize the primary antibody dilution.

Amplification

Signal amplification is carried out using the HCR™ Gold amplification platform. Each HCR™ Gold Amplifier comprises two hairpins (h1 and h2) that are snap-cooled separately before use to ensure proper folding. Introduce HCR™ Gold Amplifiers that diffuse to the cognate target where the encoded antibodies trigger growth of tethered fluorescent amplification polymers.

HCR™ Gold IF Kit Information

HCR™ Gold IF Storage Conditions and Shelf Life

Upon receiving your HCR™ Gold IF kit, please check storage conditions for each reagent. We recommend aliquoting HCR™ HiFi Encoder A and B and HCR™ Gold Amplifier to minimize freeze-thaw cycles and maximize shelf life. On the bench top, keep stock solutions on ice. Make sure all solutions are well mixed before use.

HCR™ Reagent	Storage Temperature (°C)	Storage Condition	Shelf Life (months)	Comments
HCR™ HiFi Encoder A	−20	—	6	Reactivity is specific to both the primary antibody species/isotype (mouse IgG1 or rabbit IgG) and HCR™ Gold Amplifier (X1, X2, or X3)
HCR™ HiFi Encoder B	−20	—	6	Reactivity is specific to both the primary antibody species/isotype (mouse IgG1 or rabbit IgG) and HCR™ Gold Amplifier (X1, X2, or X3)
HCR™ HiFi Antibody Buffer	4	—	12	
HCR™ HiFi Antibody Wash Buffer	4	—	12	
HCR™ Gold Amplifier†‡	−20	Shielded from light	24	Comes in two separate tubes (h1 and h2)
HCR™ Gold Amplifier Buffer‡	4	—	12	
HCR™ Gold Amplifier Wash Buffer‡	4	—	12	Provided at 4×; dilute to 1× with UltraPure H_2O before use

†Use a matching HCR™ Gold Amplifier and HCR™ HiFi Encoder (X1, X2, or X3) for a given target.
‡These reagents are not included in HCR™ HiFi Encoder Launch Edition bundles but may be conveniently purchased here.

Supplementary Materials Required to Perform HCR™ Gold IF

Reagent†	Supplier	Comments
Antibody blocking buffer	Any	Use standard antibody blocking buffer or optionally substitute with HCR™ HiFi Antibody Buffer
UltraPure H_2O	Any	Type I water
10× PBS	Any	Avoid using PBS containing calcium chloride or magnesium chloride as these can increase sample autofluorescence
10% Tween-20	Any	—
1° antibody	Any	Use one 1° antibody (must be mouse IgG1 or rabbit IgG) for each target protein
ProLong™ Gold Antifade Mountant with DAPI	ThermoFisher	This is our recommended antifade mountant but any antifade mountant, with or without DAPI, is acceptable (e.g., Fluoromount-G)

†All user-supplied reagents should be DNase and RNase-free.

Frequently Asked Questions (FAQ)

Can I multiplex with same-species/isotype primaries?

- Yes! With the HCR™ HiFi Encoder, same-species/isotype primary antibodies can be multiplexed in parallel without cross-reactivity. Each encoded antibody is uniquely paired with its corresponding HCR™ Gold Amplifier system (e.g., X1, X2, X3), allowing multiple same-species primaries to be used simultaneously in the same sample.

Are HCR™ Gold Amplifiers interchangeable between HCR™ Gold RNA-FISH kits and HCR™ Gold IF kits?

- Yes! The HCR™ Gold Amplifiers (488, 546, 647) are interchangeable between HCR™ Gold RNA-FISH and HCR™ Gold IF kits. Likewise, HCR™ Gold Amplifier Buffer and HCR™ Gold Amplifier Wash Buffer are interchangeable between HCR™ Gold RNA-FISH and HCR™ Gold IF kits.

Do carriers or amine-containing buffers interfere with HCR™ HiFi Encoding?

- Encoding is compatible with common carriers and buffers, including BSA, glycerol, Tris buffer, and preservatives such as sodium azide.

Can encoded antibodies be stored after labeling?

- Encoded antibodies are best used soon after preparation. We recommend encoding shortly before your experiment, ideally within 48 hours. However, because the stability of different primary antibodies can vary, you may wish to assess storage conditions empirically for your antibodies.

When will HCR™ Gold IF kits be available?

- HCR™ Gold IF kits will officially launch in early October, 2025. You can pre-order Launch Edition bundles of the HCR™ HiFi Encoder today, for early delivery starting the last week of September.

Which host species/isotypes and amplifiers are supported by HCR™ HiFi Encoder Launch Edition bundles?

- You can pre-order Launch Edition bundles for the following 1° antibody host species/isotypes:
  - Mouse IgG1
  - Rabbit IgG
  - Mouse IgG1 & Rabbit IgG
- Each Launch Edition bundle includes:
  - HCR™ HiFi Encoders per species/isotype selected:
    - 50 slides/25 tubes worth of Encoder for HCR™ Gold Amplifier X1
    - 50 slides/25 tubes worth of Encoder for HCR™ Gold Amplifier X2
    - 50 slides/25 tubes worth of Encoder for HCR™ Gold Amplifier X3
  - HCR™ HiFi Antibody Buffer
  - HCR™ HiFi Antibody Wash Buffer
  - Launch Edition swag

With the HCR™ HiFi Encoder Launch Edition, what additional materials do I need to perform HCR™ Gold IF?

- To perform HCR™ Gold IF with an HCR™ HiFi Encoder Launch Edition bundle, you will also need HCR™ Gold Amplifiers (X1, X2, X3 to perform a 3-plex experiment), HCR™ Gold Amplifier Buffer, and HCR™ Gold Amplifier Wash Buffer. You can conveniently purchase these here.
- You will also need to supply your own primary antibodies. Note that the host species and isotype of your antibodies must match your encoder (either mouse IgG1 or rabbit IgG). Other host species and isotypes are not compatible (e.g., a mouse IgG2a primary antibody cannot be used with a mouse IgG1 encoder).

Can I combine HCR™ Gold IF with HCR™ Gold RNA-FISH for co-detection?

- Yes! HCR™ Gold IF is compatible with HCR™ Gold RNA-FISH, as both assays are powered by the same HCR™ Gold amplification platform. An HCR™ Gold RNA-FISH/IF User Guide will be available at launch to provide guidance for co-detection workflows.

What comes in an HCR™ Gold IF kit?

- HCR™ HiFi Encoder
- HCR™ HiFi Antibody Buffer
- HCR™ HiFi Antibody Wash Buffer
- HCR™ Gold Amplifier
- HCR™ Gold Amplifier Buffer
- HCR™ Gold Amplifier Wash Buffer

Can I order a subset of these components?

- Yes! Following the launch of HCR™ Gold IF in early October, 2025, you will be able to order kits containing any subset of the above components.

What do I order for a multiplex HCR™ Gold IF experiment?

- Use your own trusted 1° antibodies (*same* host species/isotype for each target protein if desired; e.g., 10-plex experiment using Rabbit IgG 1° antibodies for all 10 target proteins)
- Order one HCR™ Gold IF kit per target protein:
    - HCR™ HiFi Encoder (each for use with a different amplifier)
    - HCR™ HiFi Antibody Buffer
    - HCR™ HiFi Antibody Wash Buffer
    - HCR™ Gold Amplifier per target (for example, amplifier X3 with label 647 for target 1, amplifier X2 with label 546 for target 2, ...)
    - HCR™ Gold Amplifier Buffer
    - HCR™ Gold Amplifier Wash Buffer
    - Note: buffers only needed in one kit
- Example 3-plex experiment:
    - HCR™ Gold IF kit for target Protein1:
        - 1° antibody: Rabbit IgG Anti-Protein1 (your own 1° antibody)
        - HCR™ HiFi Encoder: Rabbit IgG for amplifier X3
        - HCR™ HiFi Antibody Buffer (for use with all kits)
        - HCR™ HiFi Antibody Wash Buffer (for use with all kits)
        - HCR™ Gold Amplifier: X3 with label 647
        - HCR™ Gold Amplifier Buffer (for use with all kits)
        - HCR™ Gold Amplifier Wash Buffer (for use with all kits)
    - HCR™ Gold IF kit for target Protein2
        - 1° antibody: Rabbit IgG Anti-Protein2 (your own 1° antibody)
        - HCR™ HiFi Encoder: Rabbit IgG for amplifier X2
        - HCR™ Gold Amplifier: X2 with label 546
    - HCR™ Gold IF kit for target Protein3
        - 1° antibody: Rabbit IgG Anti-Protein3 (your own 1° antibody)
        - HCR™ HiFi Encoder: Rabbit IgG for amplifier X1
        - HCR™ Gold Amplifier: X1 with label 488