75: Next-Generation Multiplex IF Enabled by the Superior Amplification and Universal Antibody Compatibility of HCR™ HiFi Encoders

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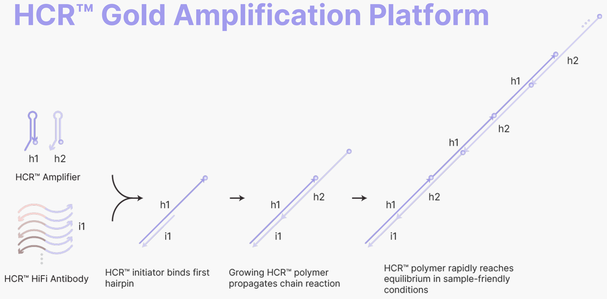
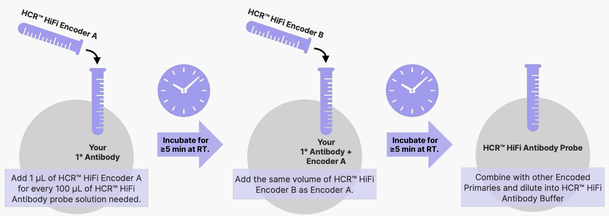
Introduction

Multiplex immunofluorescence (mIF) has become an essential tool in translational research and immuno-oncology, yet popular commercial platforms are constrained by limited primary antibody selection, fixed antibody panels, and dependence on harsh stripping or iterative labeling protocols that can compromise sample integrity and require significant time for assay development. To address these challenges, we present the HCR™ HiFi Encoder: a plug-and-play reagent that enables high performance, enzyme-free fluorescent detection using unmodified, user-supplied rabbit or mouse primary antibodies. No specialized formulations or cumbersome antibody modifications are required, enabling flexible, low-input workflows ideally suited for custom-designed panels with user selected antibodies. This approach enables rapid implementation of tumor microenvironment-focused mIF panels, such as those intended for screening of antibody-drug conjugate (ADC) targets and quantification of emerging immune biomarkers.

HCR™ Gold Amplification Platform



HCR™ HiFi Encoder Workflow



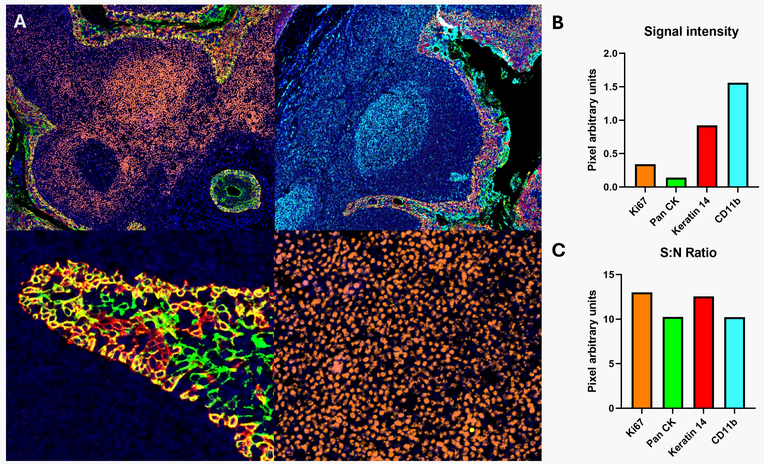
Antibody	Clone	Host	Isotype	Channel	Fluor
Ki67	SP6	Rabbit	IgG	X2	AF546
Pan CK	AE1/AE3	Mouse	IgG1 Kappa	X8	AF594
Keratin 14	E7W6V	Rabbit	IgG	X3	AF647
CD11b	EPR1344	Rabbit	IgG	X1	AF488
Pan CK	AE1/AE3	Mouse	IgG1 Kappa	X4	AF750
Trop2	CB11	Rabbit	IgG	X3	AF647

Antibody clones evaluated with the HiFi Encoder Workflow

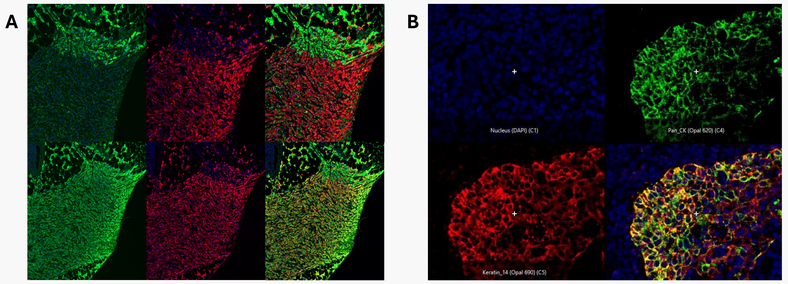
Methods

The HCR™ HiFi Encoder binds with high affinity to the Fc domain of unmodified primary antibodies, forming a stable 1:1 complex without compromising antigen recognition. This encoding process is fully compatible with commonly used antibody buffers and requires as little as 0.5 µg of primary antibody input. HiFi-encoded antibodies were applied to FFPE human tissue sections and detected with fluorophore-labeled HCR™ Gold amplifiers. Multiplexing was achieved by assigning orthogonal HCR™ initiators to each unmodified antibody, allowing simultaneous detection of multiple targets within a single sample. We then employed the assay to simultaneously examine relevant immune-oncology markers (Ki67, CD11b, Keratin 14, Trop-2, and Pan-cytokeratin) in patient breast and colorectal cancer tumor microenvironments.

Results: HiFi Encoders allow for rapid multiplex IF of FFPE tissues

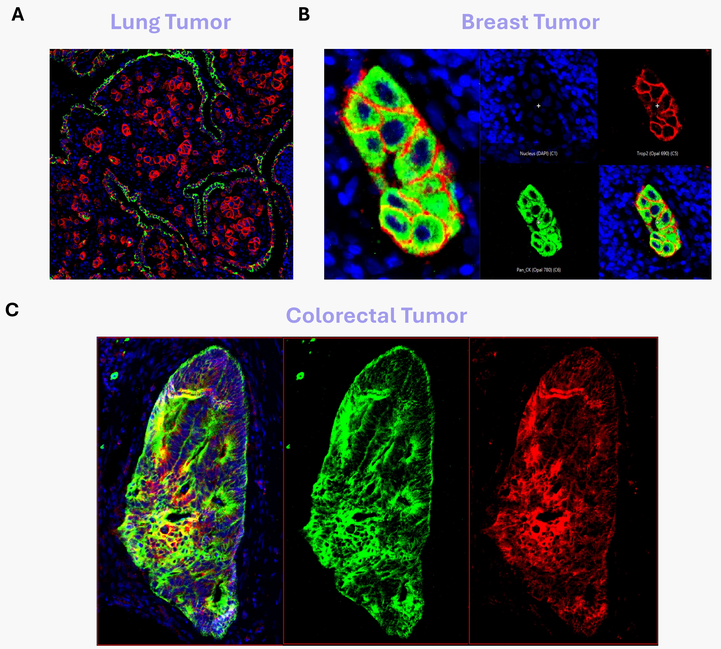


HCR™ HiFi Encoders for cell profiling. Encoders allow for staining of multiple markers in a single-step protocol. A) Human tonsil stained with antibodies for Ki67 (orange), CD11b (cyan), Pan-Cytokeratin (green) and Keratin 14 (red). B) Varied signal intensities are observable across different antigen densities. C) High signal-to-noise ratios for multiple antibody clones screened.



Traditional sequential IF staining is hindered by epitope accessibility. A significant challenge with protocols requiring multiple rounds of antibody staining is the possibility of blocking epitopes when two or more targets are co-expressed. A) HER2 (Green) and TROP2 (Red) seem to be mutually blocked when staining HER2 first (Top) but not when staining TROP2 first (bottom). B) Simultaneous multiplexing enabled by HCR™ HiFi Encoders with a single antibody cocktail allows for assessment of co-expressed markers. This is the case for Pan CK (green) and Keratin 14 (red), showing both single and double positive cells for both markers in a human breast cancer tumor specimen.

Results: HiFi Encoders work readily with on-hand primary antibodies



HCR™ HiFi Encoders enable rapid IF multiplexing with a variety of off-the-shelf antibodies. HiFi Encoders can be applied to most antibodies that researchers or pathologists have available and previously validated. This allows for rapid construction of multiplex panels and assessment of co-expression of novel or emerging antigen targets in clinical tissue samples. Representative images show human lung and colorectal tumor samples were stained with encoded antibodies specific for Pan-CK (Green) and Trop-2 (Red) using Encoders. A) Lung tumor showing cytokeratin in epithelial layer, while Trop-2 expression is found in cytokeratin low cancer cells. B) Breast tumor showing co-expression of Pan-CK and Trop-2. C) Colorectal tumor showing heterogeneous patterns of Pan-CK and Trop-2 co-expression.

Conclusions

- HCR™ HiFi Encoders enable rapid and flexible multiplexing of off-the-shelf antibodies with no requirement for carrier-free or glycerol-free antibody storage medias.
- Single-step staining of a multiplex IF panel via HiFi Encoders allows for rapid assessment of co-expressing antigens in FFPE-based tumor microenvironment studies.

Questions?

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