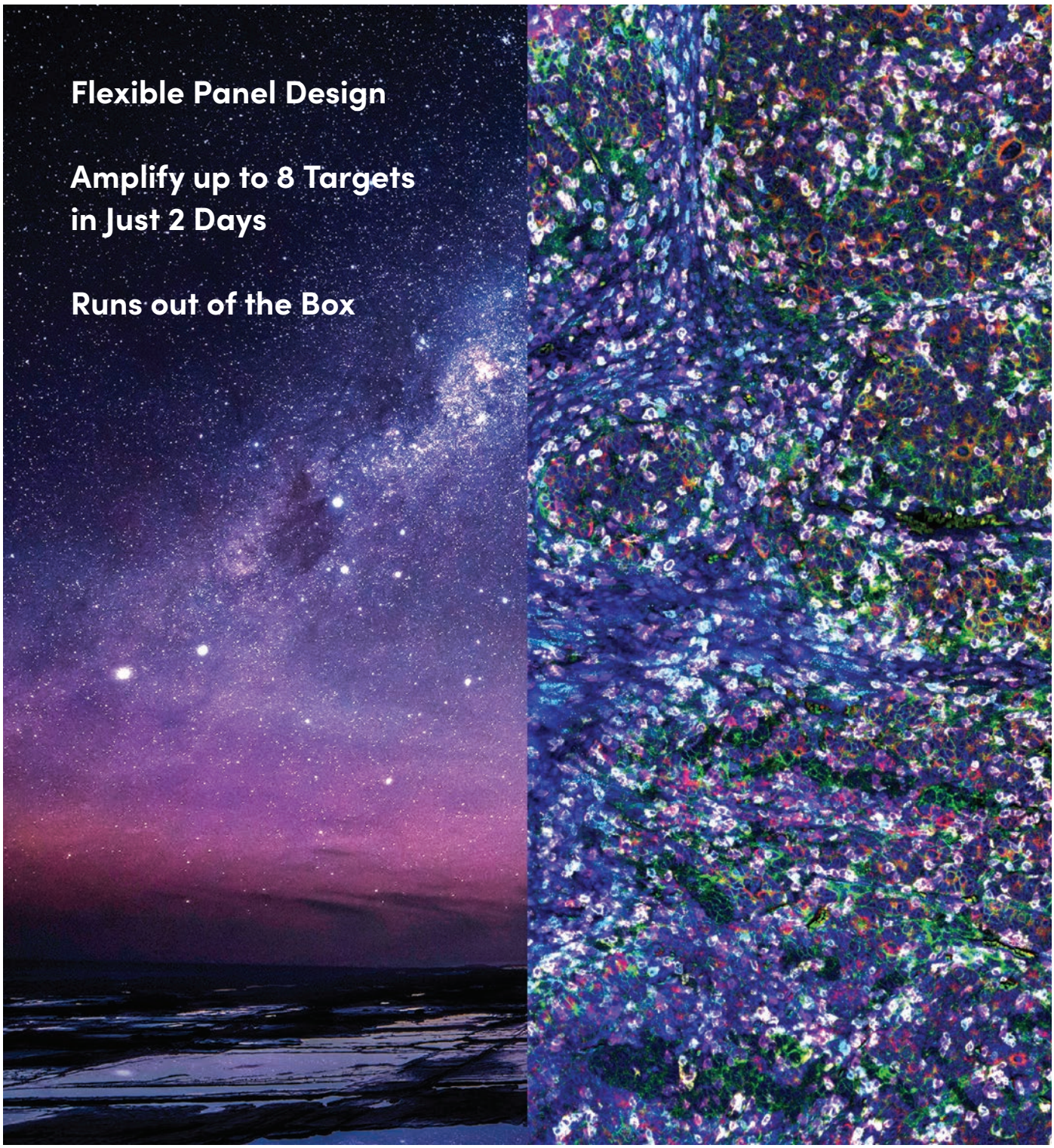
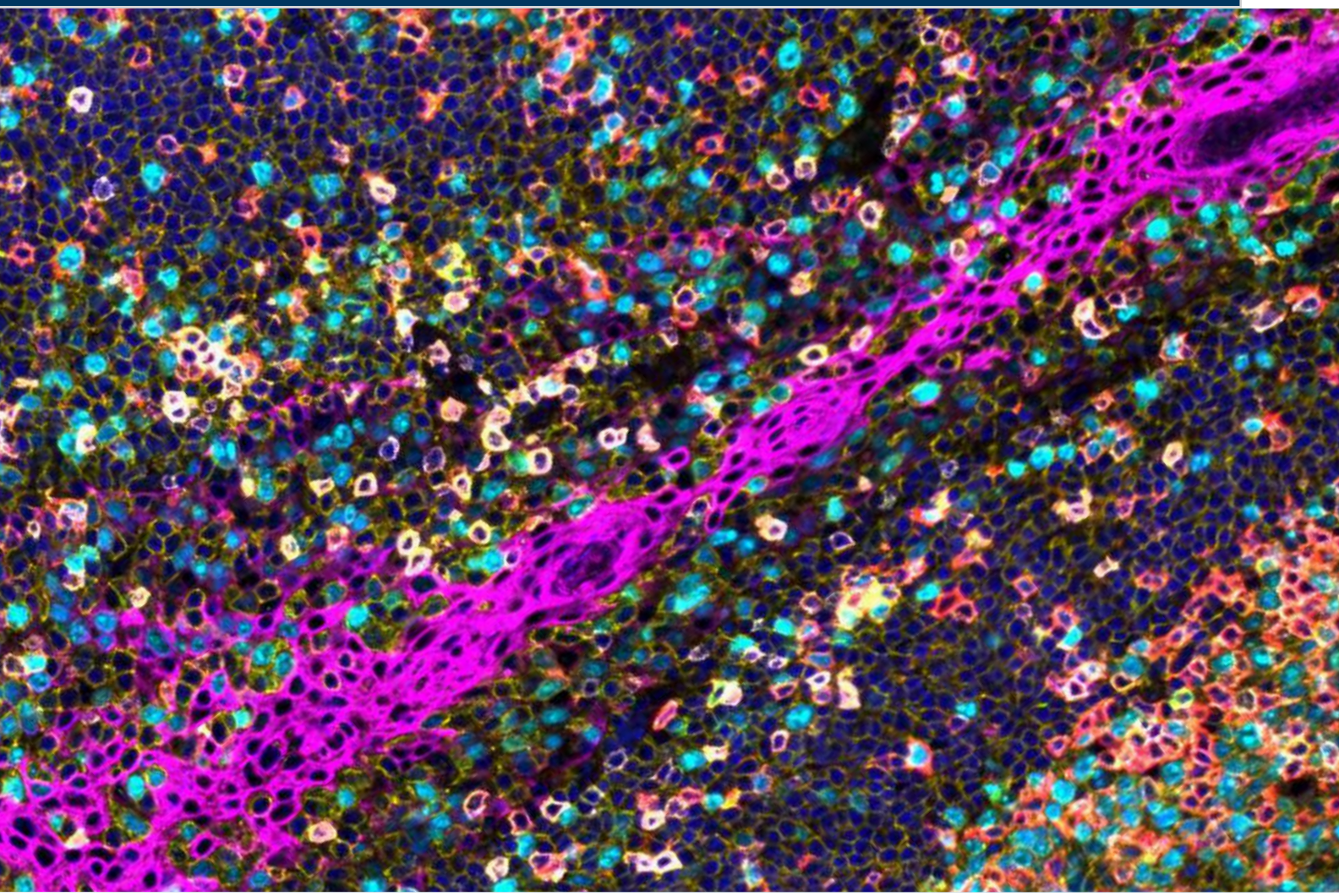
Cell Signaling TECHNOLOGY®

OZYME
Des femmes et des hommes au service de vos recherches

SignalStar™ Multiplex IHC for Spatial Biology





Spatial Biology Has A New Star

Current spatial biology and multiplex immunohistochemistry (mIHC) technologies limit progress in discovering mechanisms of disease due to the time-consuming methods needed for panel optimization, validation, and analysis.

With SignalStar technology, you can detect multiple targets simultaneously with flexible, highly-validated antibody panels—accelerating data generation and letting you focus on results.

Image Above: SignalStar multiplex immunohistochemical analysis of paraffin-embedded human tonsil using Granzyme B (D6E9W) & CO-0009-647 SignalStar Oligo-Antibody Pair #37369 (white), Phospho-SLP-76 (Ser376) (E3G9U) & CO-0018-488 SignalStar Oligo-Antibody Pair #34567 (green), CD3ε (D7A6E) & CO-0001-488 SignalStar Oligo-Antibody Pair #92856 (pink), CD20 (E7B7T) & CO-0011-594 SignalStar Oligo-Antibody Pair #54189 (yellow), CD8α (D8A8Y) & CO-0004-594 SignalStar Oligo-Antibody Pair #19166 (orange), ICOS (D1K2T) & CO-0027-647 SignalStar Oligo-Antibody Pair #24147 (red), Ki-67 (8D5) & CO-0014-750 SignalStar Oligo-Antibody Pair #56398 (cyan), Pan-Keratin (C11) & CO-0003-750 SignalStar Oligo-Antibody Pair #97227 (magenta), and ProLong Gold Antifade Reagent with DAPI #8961 (blue). All fluorophores have been assigned a pseudocolor, as indicated. Staining was performed on the BOND RX Fully Automated Research Stainer by Leica Biosystems.

The Many Benefits of SignalStar

GENERATE DATA IN JUST 2 DAYS

Timelines for panel optimization and validation for other multiplex technologies can take weeks to months. SignalStar technology eliminates that time with optimized, ready-to-go panels. Select targets from a menu of IHC-validated antibodies that work out of the box in FFPE tissues.

REDESIGN PANELS EASILY

Many solutions lack the flexibility to evolve and adapt to changing research needs. SignalStar panels give you the flexibility to easily change targets and redesign panels as your needs change. Protocols are validated and optimized to work out of the box.

AMPLIFY YOUR SIGNAL

Unlike many other solutions, SignalStar panels offer amplification. See more than you ever have before and detect targets with low levels of expression.

USE YOUR EXISTING INSTRUMENTATION

Stable fluorescent signals with distinct emission peaks make SignalStar panels compatible with your existing imaging instrumentation.

RESULTS YOU CAN TRUST

Every antibody used in a SignalStar panel has been independently validated for use in its intended application and guaranteed to perform as expected just like all CST® antibodies—to provide you with reliable, reproducible results, the first time and every time. You'll also be taking advantage of the most cited antibodies on the market.*

ELIMINATE ANTIBODY CYCLING

Add all your antibodies at once without having to worry about epitope loss or masking.

DESIGN EXPERIMENTS FASTER

Our online, easy-to-use SignalStar Multiplex IHC Panel Builder does the design work so you don't have to.

*Of the 10 antibodies most often cited in peer-reviewed publications, six are from CST—and more than one-third of the top 100-cited antibodies are from CST, including the top-cited primary and secondary antibodies. Source: *CiteAb Blog, "What were the top 100 research antibodies of 2022?"* July, 2023.

Results up to 70% Faster

SignalStar technology is up to 70% faster from sample to imaging than other mIHC methods. Adding an autostainer to your workflow eliminates another 12 hours of hands-on time.

For an 8-Plex Assay		
	CST® SignalStar technology	**Other mIHC technologies**
Panel optimization time	0 days*	Up to 6 months
Protocol time	2 days	7 days

*Works out-of-the-box with most FFPE tissues.

Easily design your experiments in our online SignalStar Multiplex IHC Panel Builder, and select targets from a menu of validated antibodies that work right out of the box. **CST ships your customized kits within 2 business days.**

You can also take advantage of our expert Scientific Support team to answer questions anytime.

Plex Your Way

Define your plex, run the easy-to-use manual or BOND RX protocols, then image and analyze using your existing equipment and software. SignalStar antibodies and fluorophores are interchangeable across panels, giving you the flexibility to change targets within panels anytime.

- Select from a growing menu of CST oligo-conjugated, IHC-validated primary antibodies
- Stain 10 slides per panel—we provide all the reagents and materials you need
- Image up to 8 protein markers utilizing fluorescent channels of 488, 594, 647, and 750 nm
- Universal protocols for manual or BOND RX autostainer

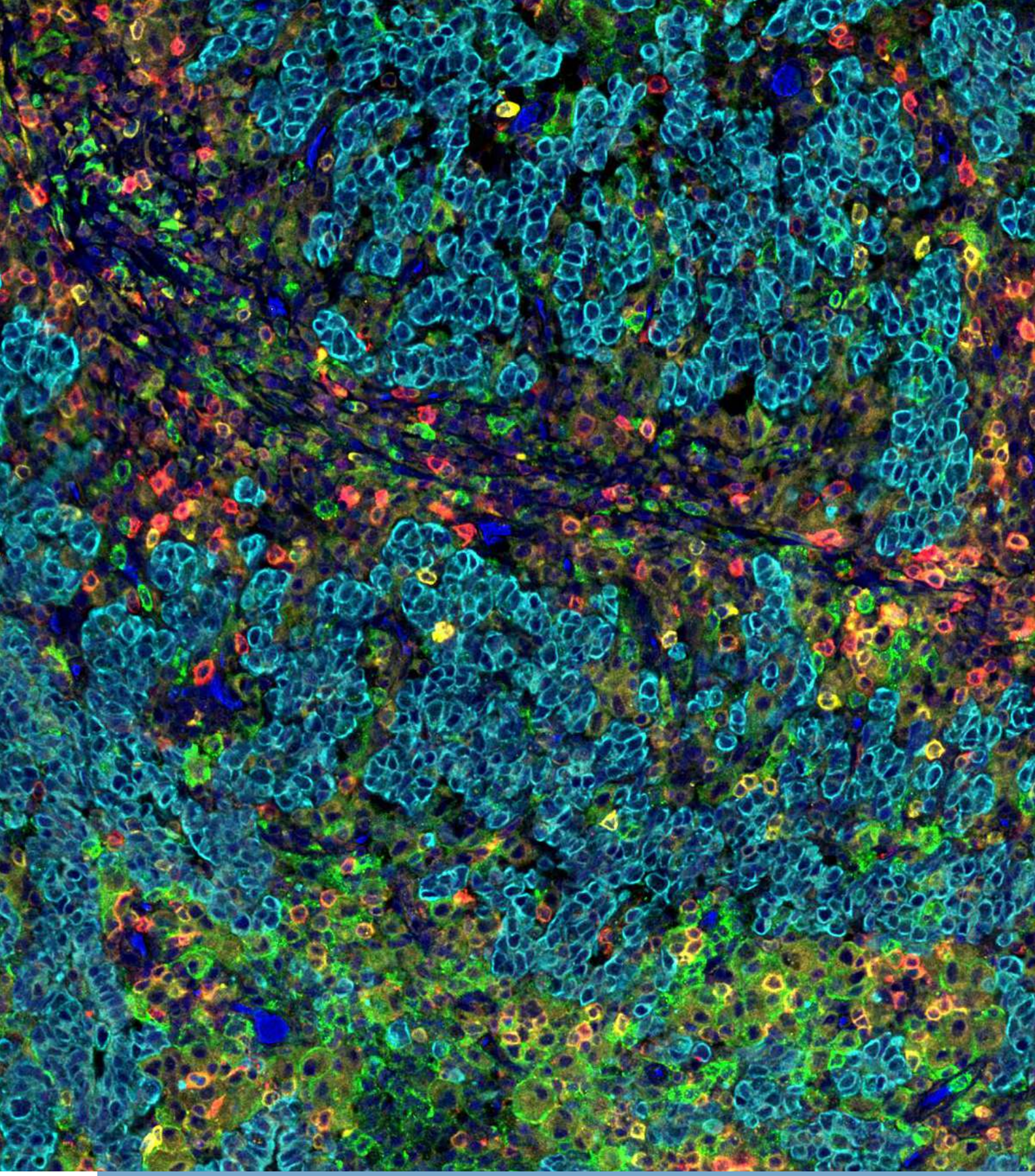
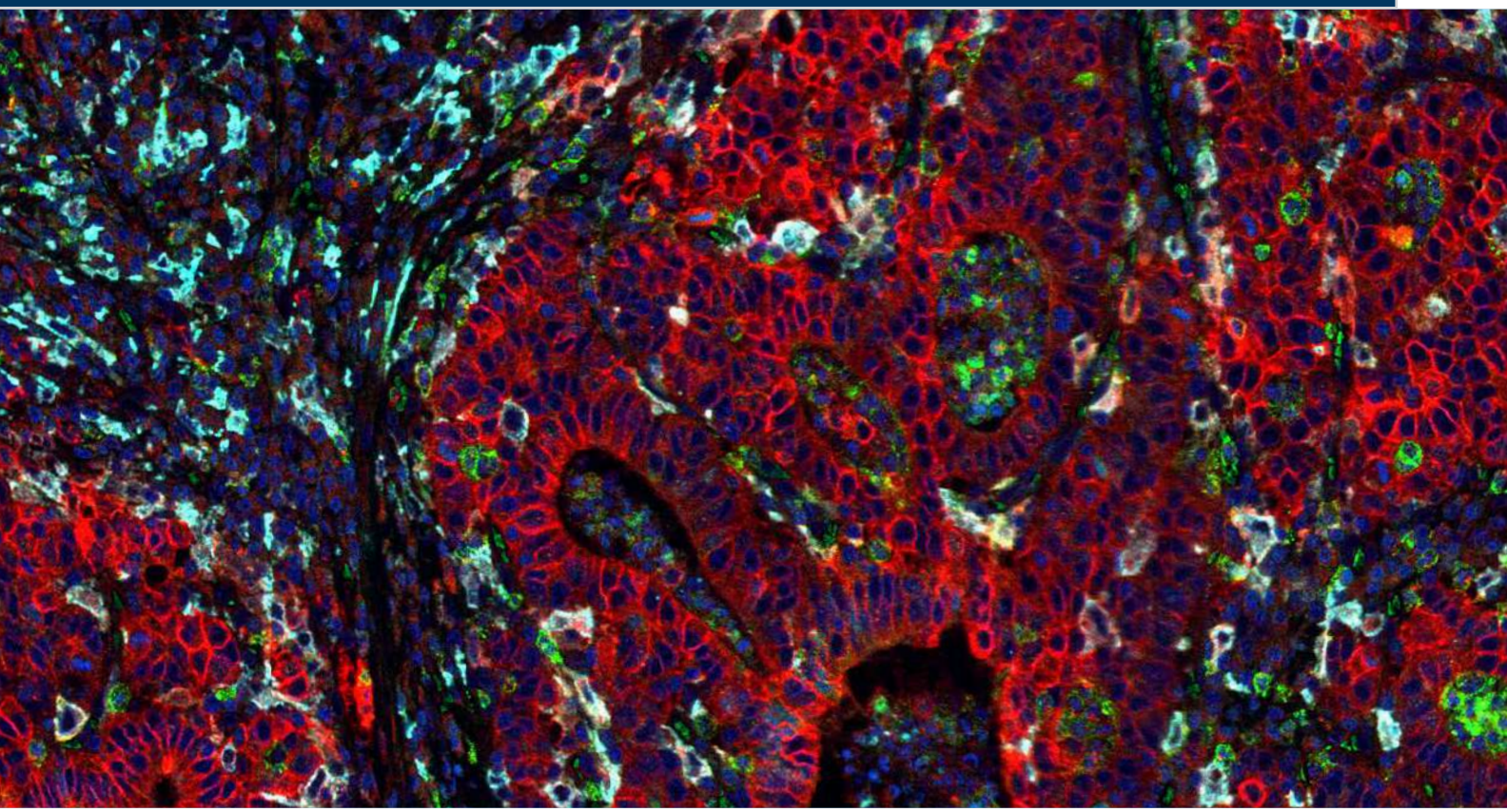


Image Above: SignalStar™ multiplex immunohistochemical analysis of paraffin-embedded human gastric adenocarcinoma using ICOS (D1K2T) & CO-0027-647 SignalStar Oligo-Antibody Pair #24147 (red), CD16 (D1N9L) & CO-0031-488 SignalStar Oligo-Antibody Pair #29967 (green), Phospho-SLP-76 (Ser376) (E3G9U) & CO-0018-594 SignalStar Oligo-Antibody Pair #53299 (yellow), Pan-Keratin (C11) & CO-0003-750 SignalStar Oligo-Antibody Pair #97227 (cyan), and ProLong Gold Antifade Reagent with DAPI #8961 (blue). All fluorophores have been assigned a pseudocolor, as indicated.



How SignalStar Multiplex IHC Works

IMAGING ROUND 1

- FFPE tissue sections undergo deparaffinization, rehydration, and antigen retrieval (A) followed by the addition of up to 8 oligo-conjugated antibodies (B).
- All of the antibodies in your plex size of choice (3-8 oligo-conjugated antibodies) are added in one primary incubation step. Next, complementary oligonucleotides with fluorescent dyes (488, 594, 647, and 750 nm) amplify the signal of up to 4 oligo-conjugated antibodies in the first round of imaging (C-D).

IMAGING ROUND 2

- If your plex size is greater than 4, the oligonucleotides and fluorophores are gently removed (E), and a second round of complementary oligonucleotides with fluorescent dyes amplify the signal of up to 4 additional oligo-conjugated antibodies (F).
- After the second imaging round is complete (G), the two images can be aligned computationally to generate the full up to 8-plex image.

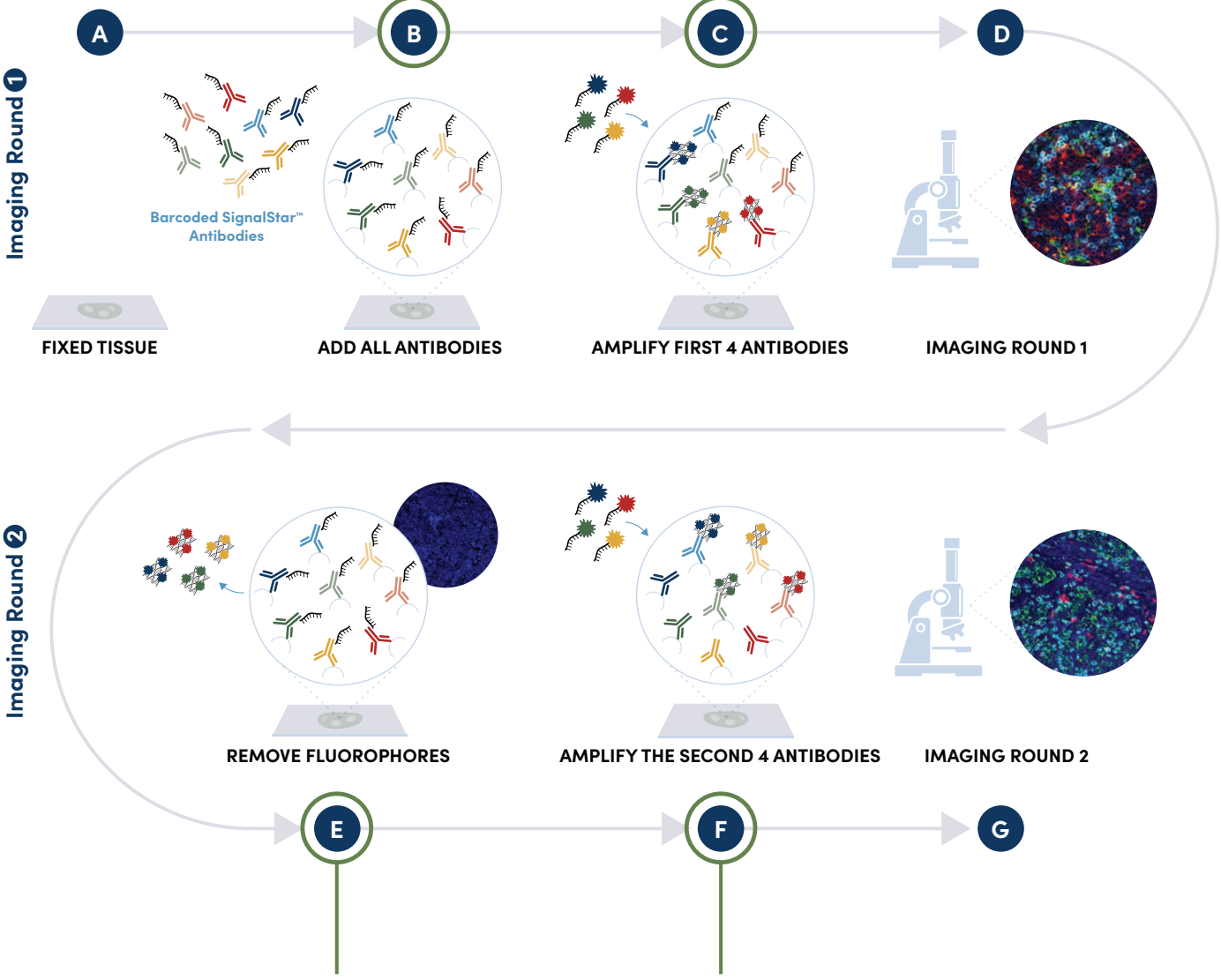
Image Above: SignalStar multiplex immunohistochemical analysis of paraffin-embedded human gastric adenocarcinoma using CD163 (D6U1J) & CO-0022-750 SignalStar Oligo-Antibody Pair #71043 (cyan), CD16 (D1N9L) & CO-0031-488 SignalStar Oligo-Antibody Pair #29967 (green), PD-L1 (E1L3N®) & CO-0005-647 SignalStar Oligo-Antibody Pair #52085 (red), and ProLong Gold Antifade Reagent with DAPI #8961. All fluorophores have been assigned a pseudocolor, as indicated.

SKIP LABORIOUS, TEDIOUS STEPS

All antibodies are added simultaneously.

AMPLIFIED SIGNAL

All target signals are amplified, including low abundance targets.



REMOVE DYE WITHOUT RISK

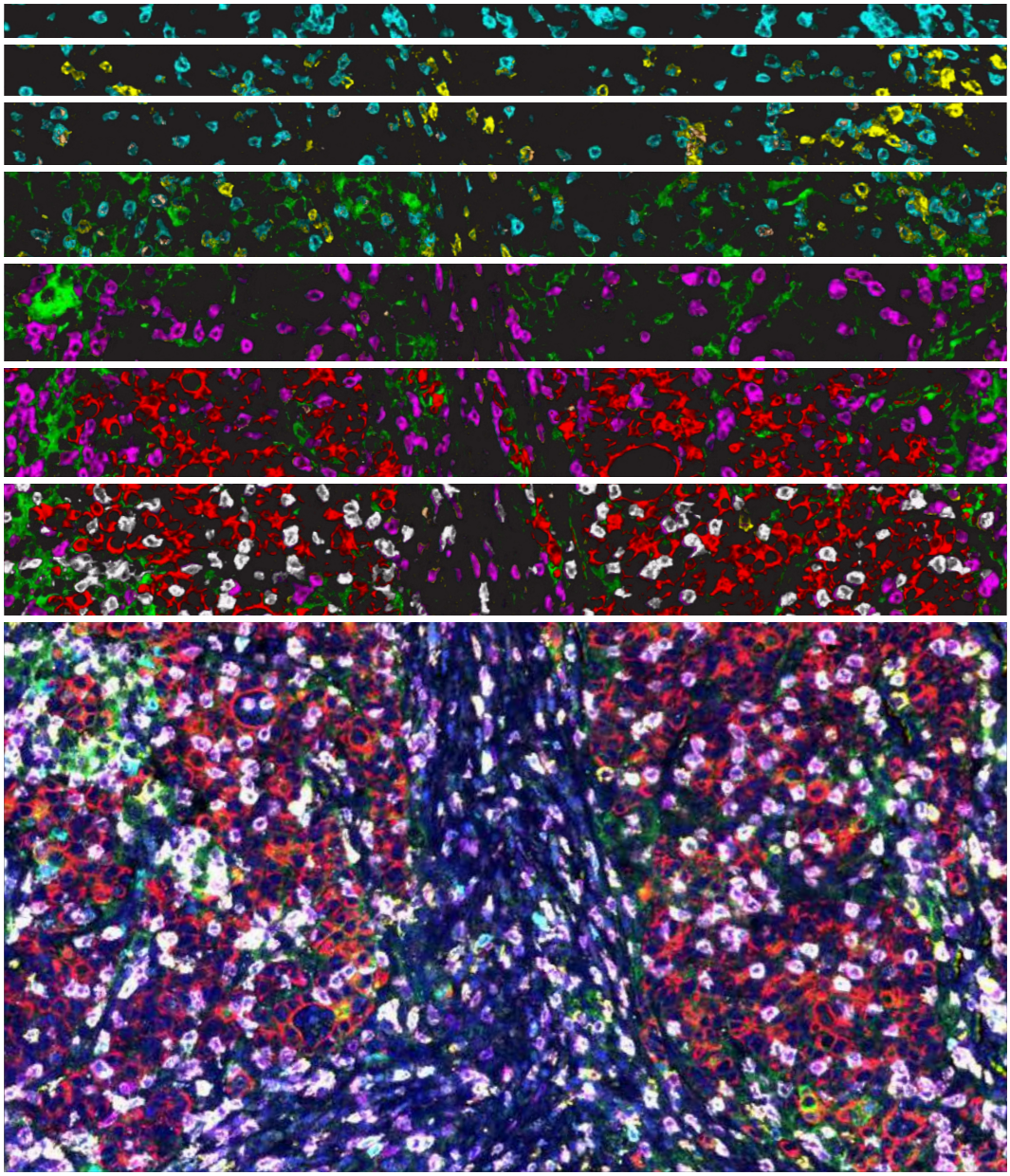
Unique technology gently removes amplified fluorescent signal between imaging rounds with no impact on antibody binding or tissue damage.

COMPLETE IMAGING IN 2 DAYS

Two rounds of up to 4 non-cross-reacting oligo-conjugated antibodies are amplified and imaged.

8-Plex in FFPE Tissue Samples

Confidently analyze limited, precious FFPE tissue samples using highly-sensitive assays that give you the signal you need the first time. **SignalStar assays enable you to amplify the fluorescent signal of up to 8 proteins in a single tissue.**



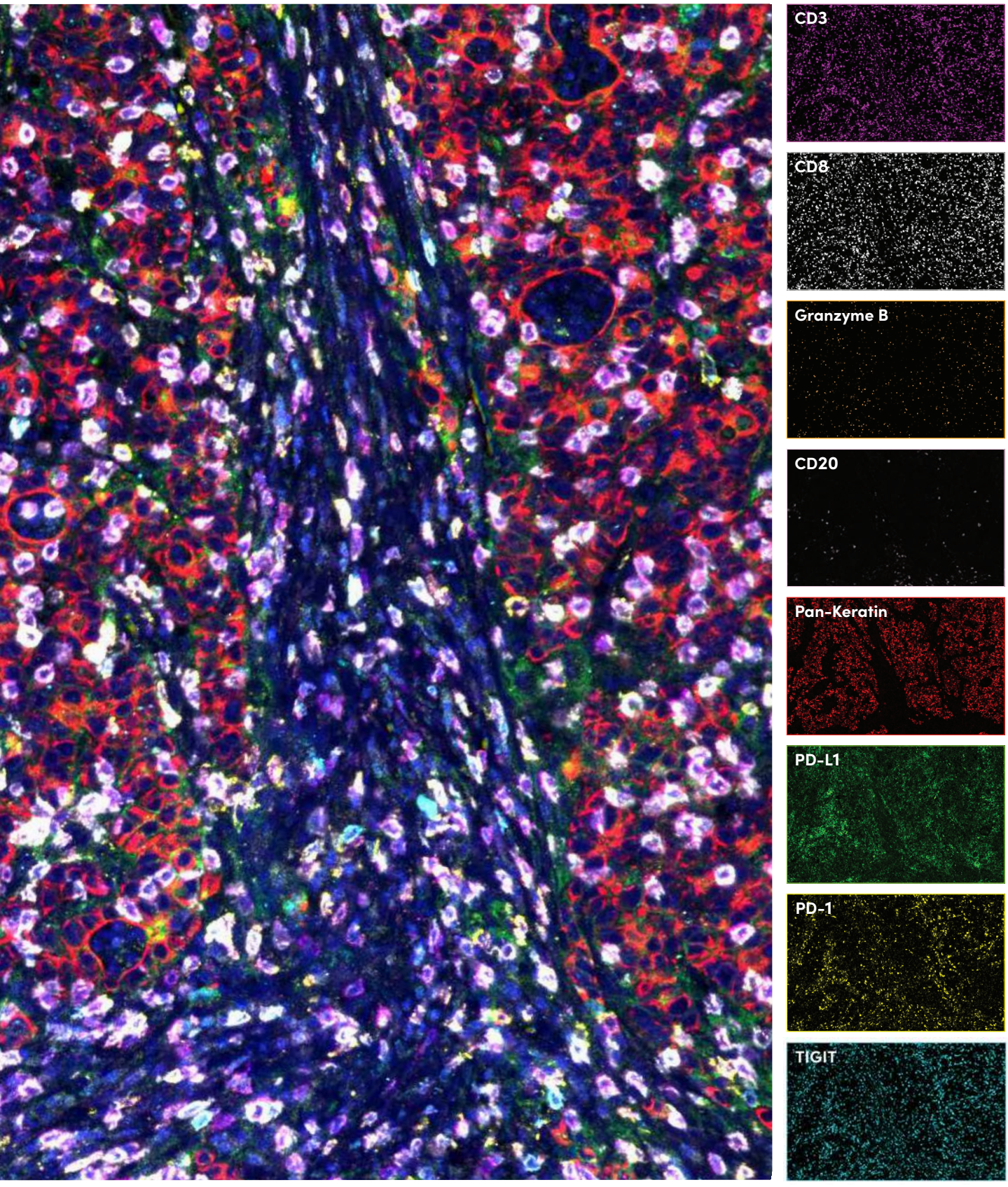
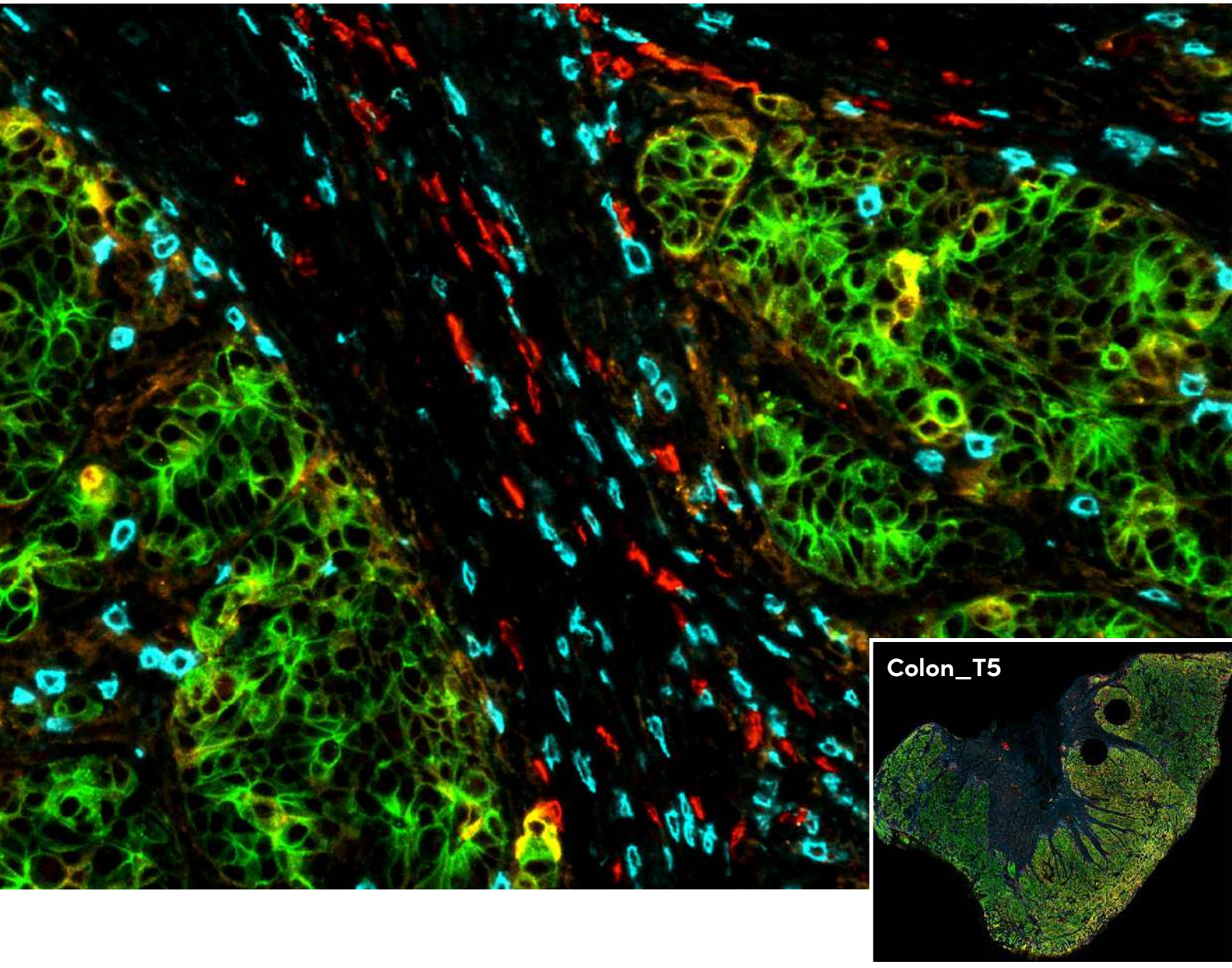


Image Above: SignalStar multiplex immunohistochemical analysis of paraffin-embedded human gastric adenocarcinoma using Pan-Keratin (C11) & C0-0003-488 SignalStar Oligo-Antibody Pair #63566 (red), PD-L1 (E1L3N) & C0-0005-488 SignalStar Oligo-Antibody Pair #85646 (green), PD-1 (Intracellular Domain) (D4W2J) & C0-0008-594 SignalStar Oligo-Antibody Pair #35347 (yellow), Granzyme B (D6E9W) & C0-0009-594 SignalStar Oligo-Antibody Pair #15194 (orange), TIGIT (E5Y1W) & C0-0002-647 SignalStar Oligo-Antibody Pair #18288 (cyan), CD20 (E7B7T) & C0-0011-647 SignalStar Oligo-Antibody Pair #36775 (pink), CD3ε (D7A6E) Conjugate & C0-0001-750 SignalStar Oligo-Antibody Pair #51754 (magenta), CD8ε (D8A8Y) & C0-0004-750 SignalStar Oligo-Antibody Pair #62750 (white), and ProLong Gold Antifade Reagent with DAPI #8961 (blue). Representative individual and co-registered 8-plex images are included. All fluorophores were assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer.

Reproducible Every Time

We validate our antibodies in the SignalStar assay and guarantee them to work as expected. Every SignalStar antibody is validated against the chromogenic gold standard, and is consistently reproducible across replicates.



Percent Positive Cells / Field of View			
Percent Positive Cells	**4-plex Duplicates**		**DAB**
Pan-Keratin	49.0%	54.0%	59.0%
PD-L1	19.3%	23.4%	25.6%
CD20	1.3%	1.5%	2.4%
CD8	8.7%	10.7%	8.1%

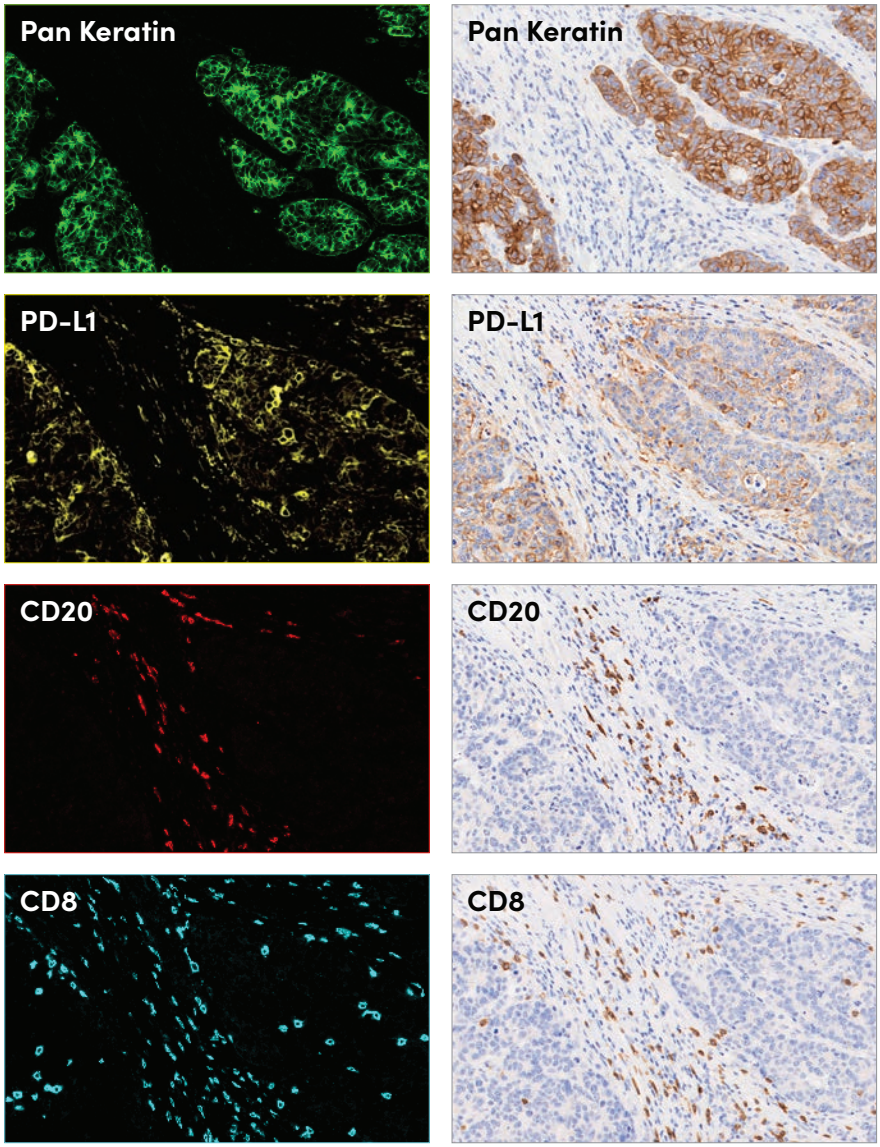


Image Left: SignalStar multiplex immunohistochemical analysis of paraffin-embedded human colorectal adenocarcinoma using Pan-Keratin (C11) & C0-0003-488 SignalStar Oligo-Antibody Pair #63566 (green), PD-L1 (E1L3N®) & C0-0005-594 SignalStar Oligo-Antibody Pair #28249 (yellow), CD20 (E7B7T) & C0-0011-647 SignalStar Oligo -Antibody Pair #36775 (red), and CD8a (D8A8Y) & C0-0004-750 SignalStar Oligo-Antibody Pair #62750 (cyan). Representative individual and 4-plex images are included. All fluorophores were assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer. Percent positive cells were quantified in matched regions of interest from serial sections for the SignalStar assay (n=2) compared to the chromogenic.

Flexible, Ready-to-Use Panels

SignalStar technology stains consistently regardless of the panel you design in the SignalStar Multiplex IHC Panel Builder, and panels are ready to use with optimized protocols. Protocols also demonstrate reproducibility across different antibody combinations, experiments, and imaging instruments.

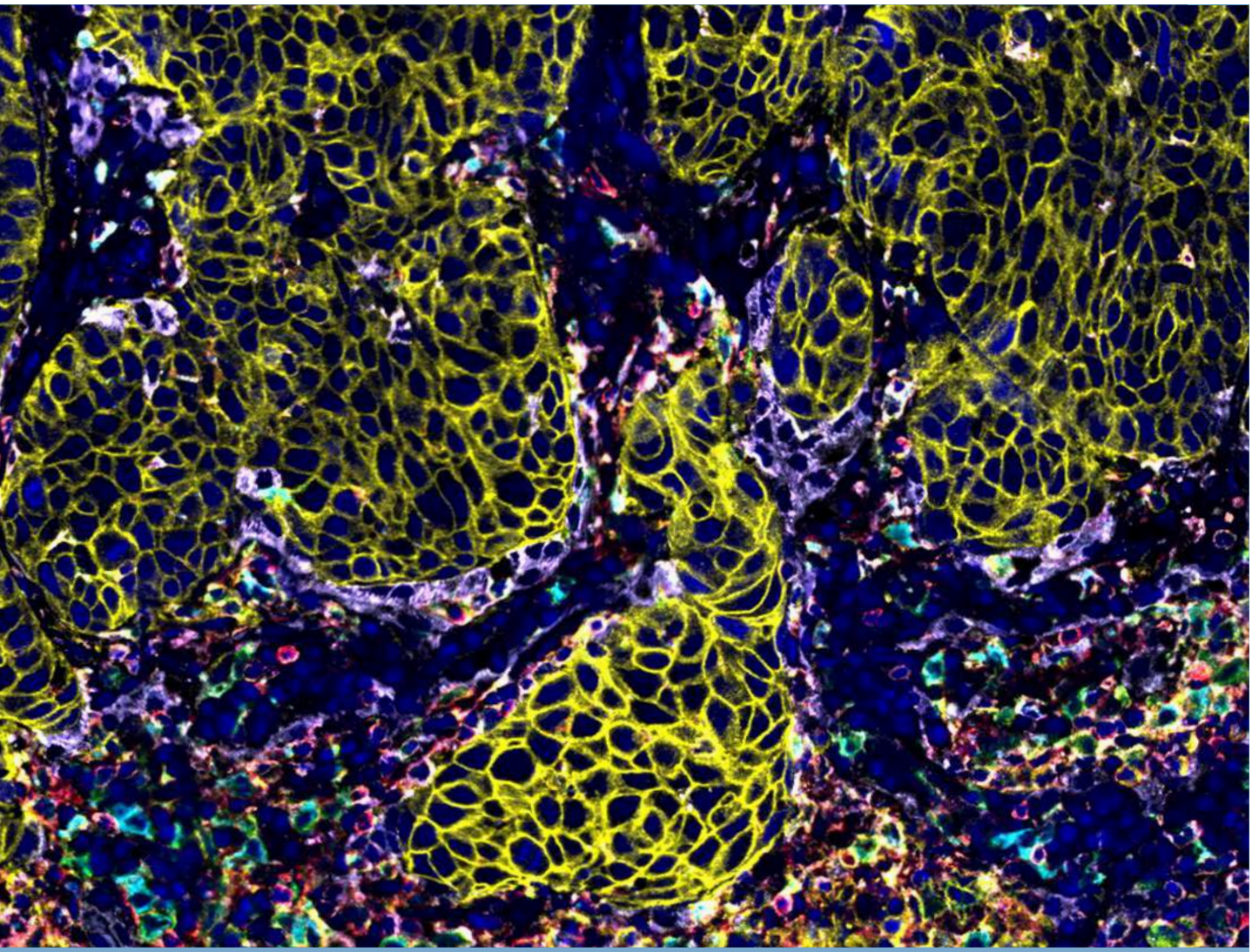


Image Above: SignalStar multiplex immunohistochemical analysis of paraffin-embedded human squamous cell lung carcinoma using CD163 (D6U1J) & CO-0022-488 SignalStar Oligo-Antibody Pair #35238 (green), PD-L1 (E1L3N®) & CO-0005-594 SignalStar Oligo-Antibody Pair #28249 (yellow), PD-1 (Intracellular Domain) (D4W2J) & CO-0008-647 SignalStar Oligo-Antibody Pair #56837 (red), CD11c (D3V1E) & CO-0017-647 SignalStar Oligo-Antibody Pair #96411 (magenta), CD68 (D4B9C) & CO-0007-750 SignalStar Oligo-Antibody Pair #91044 (cyan), HLA-DRA (E9R2Q) & CO-0023-488 SignalStar Oligo-Antibody Pair #92941 (pink), CD3ε (D7A6E) & CO-0001-594 SignalStar Oligo-Antibody Pair #84634 (orange), Pan-Keratin (C11) & CO-0003-750 SignalStar Oligo-Antibody Pair #97227 (white), and ProLong Gold Antifade Reagent with DAPI #8961 (blue). All fluorophores have been assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer.

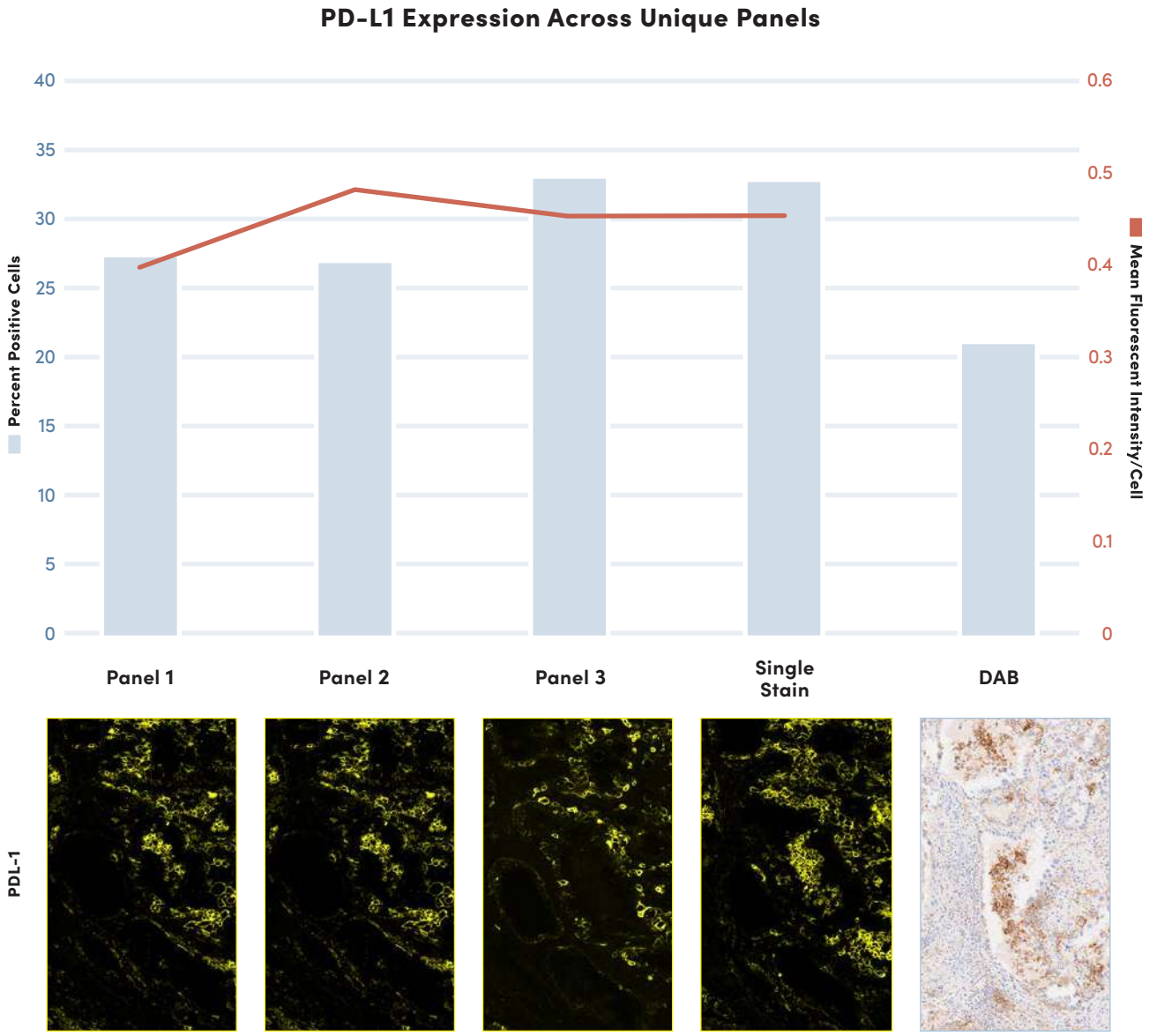
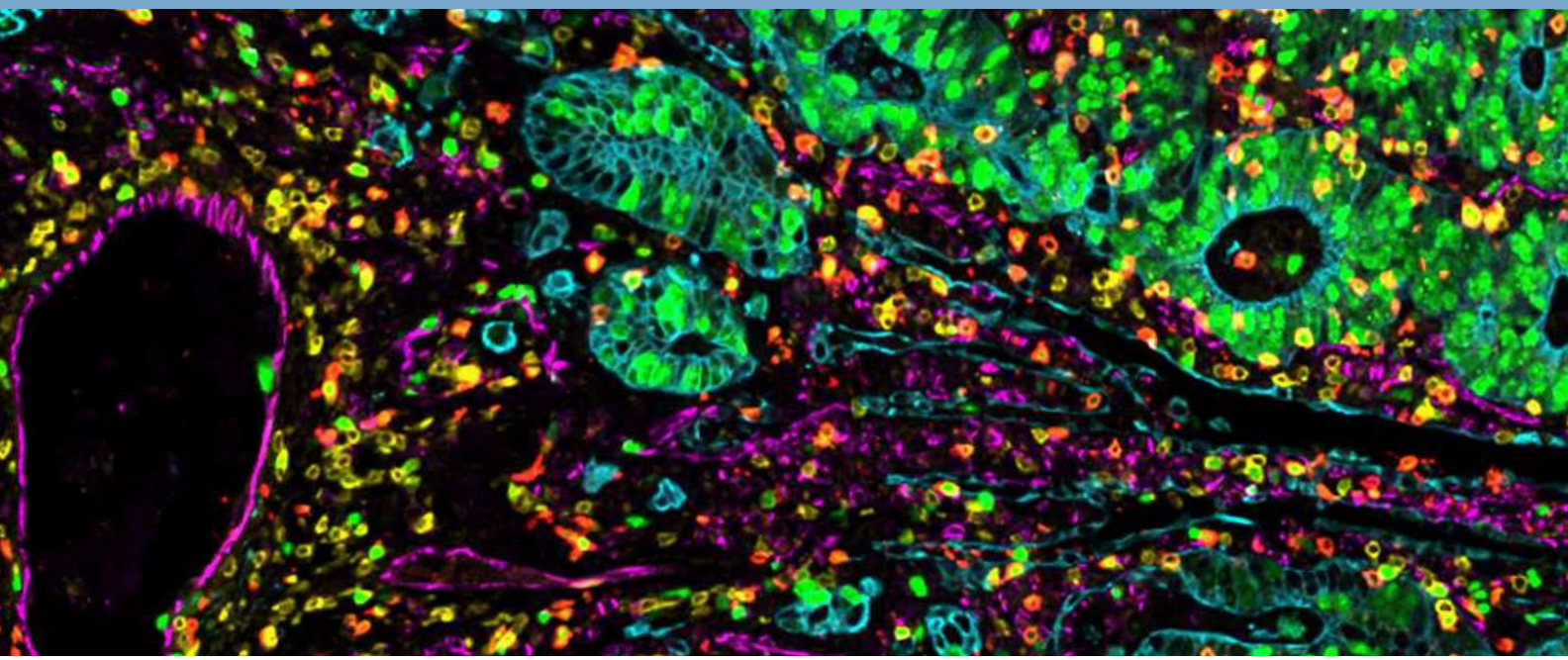


Figure Above: SignalStar multiplex immunohistochemical analysis of paraffin-embedded human medullary thyroid carcinoma within a tissue microarray. **Panel 1:** PD-L1 (E1L3N) & C0-0005-647 SignalStar Oligo-Antibody Pair #52085 (yellow), PD-1 (Intracellular Domain) (D4W2J) & C0-0008-594 SignalStar Oligo-Antibody Pair #35347 (not shown), CD68 (D4B9C) & C0-0007-488 SignalStar Oligo-Antibody Pair #73071 (not shown), and CD3ε (D7A6E) & C0-0001-750 SignalStar Oligo-Antibody Pair #51754 (not shown). **Panel 2:** PD-L1 (E1L3N) & C0-0005-647 SignalStar Oligo-Antibody Pair #52085 (yellow), PD-1 (Intracellular Domain) (D4W2J) & C0-0008-594 SignalStar Oligo-Antibody Pair #35347 (not shown), CD68 (D4B9C) & C0-0007-488 SignalStar Oligo-Antibody Pair #73071 (not shown), and CD8 (D8A8Y) & C0-0004-750 SignalStar Oligo-Antibody Pair #62750 (not shown). **Panel 3:** PD-L1 (E1L3N) & C0-0005-647 SignalStar Oligo-Antibody Pair #52085 (yellow), PD-1 (Intracellular Domain) (D4W2J) & C0-0008-594 SignalStar Oligo-Antibody Pair #35347 (not shown), Ki-67 (8D5) & C0-0014-488 SignalStar Oligo-Antibody Pair #89034 (not shown), and CD8α (D8A8Y) & C0-0004-750 SignalStar Oligo-Antibody Pair #62750 (not shown). All fluorophores were assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer. Percent positive PD-L1 cells were quantified in matched regions of interest from serial sections for three unique SignalStar 4-plex panels compared to single-plex SignalStar staining using PD-L1 (E1L3N) & C0-0005-647 SignalStar Oligo Antibody Pair #52085 (yellow) and chromogenic staining using PD-L1 (E1L3N) XP® Rabbit mAb #13684.

Image Below: SignalStar™ multiplex immunohistochemical analysis of paraffin-embedded human colorectal adenocarcinoma using Ki-67 (8D5) & CO-0014-750 SignalStar Oligo-Antibody Pair #56398 (green), Pan-Keratin (C11) & CO-0003-488 SignalStar Oligo-Antibody Pair #63566 (cyan), CD31 (PECAM-1) (89C2) & CO-0028-488 SignalStar Oligo-Antibody Pair #83823 (magenta), CD3ε (D7A6E) & CO-0001-594 SignalStar Oligo-Antibody Pair #84634 (yellow), and CD8α (D8A8Y) & CO-0004-647 SignalStar Oligo-Antibody Pair #66676 (red). All fluorophores have been assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer.



Guaranteed Product Quality

Experienced CST scientists who understand the underlying biology develop, test, and rigorously validate our products across multiple applications to ensure specificity. If any product fails to meet our stringent standards, we won't sell it. This rigorous product quality ensures you'll always get the lot-to-lot and assay-to-assay reproducibility you need to keep your research moving forward.

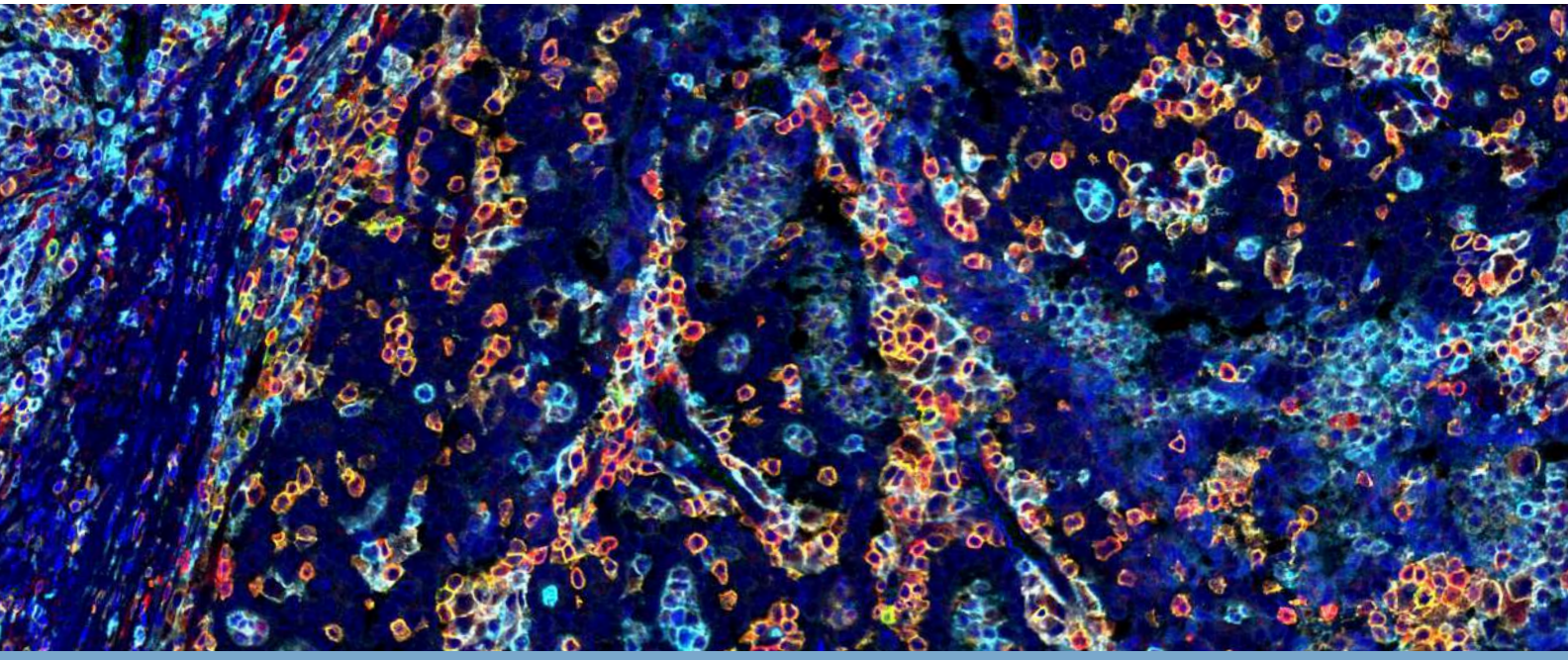
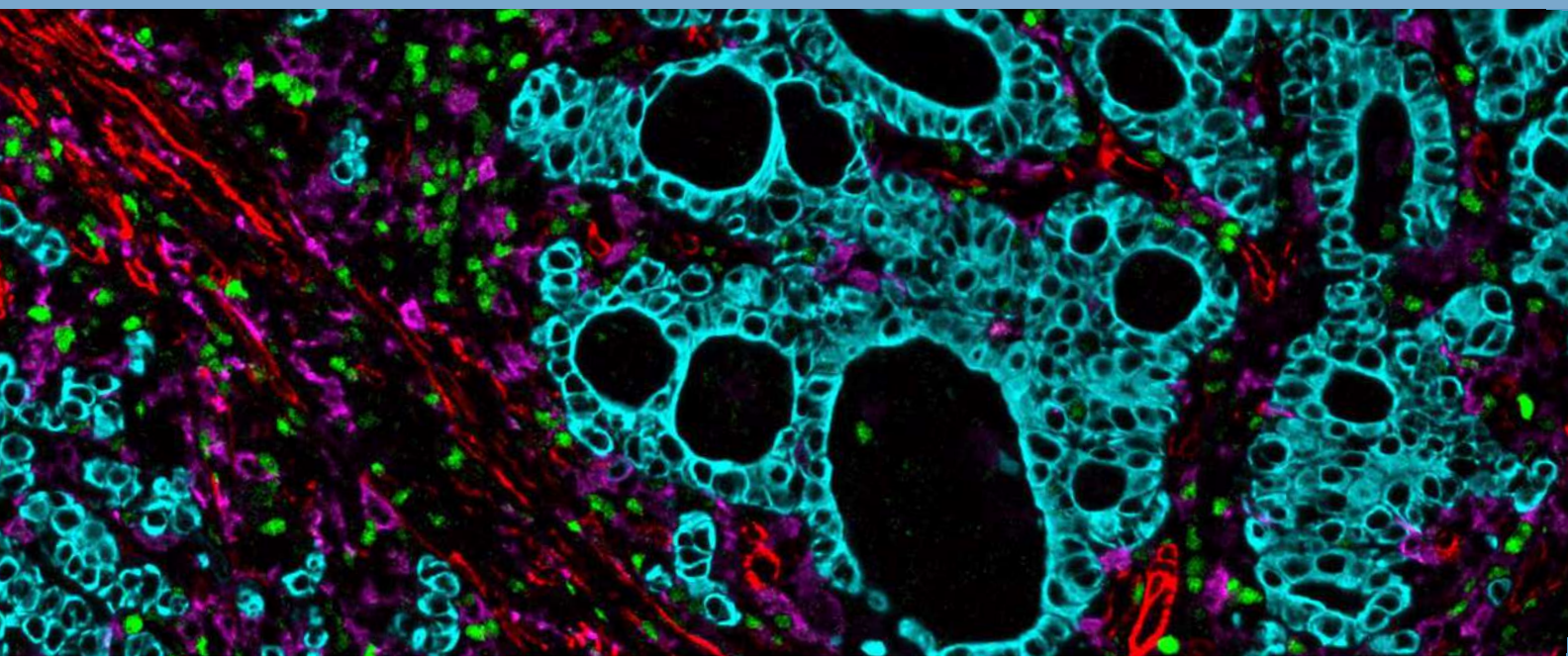


Image Above: SignalStar multiplex immunohistochemical analysis of paraffin-embedded human gastric adenocarcinoma using ICOS (D1K2T) & CO-0027-488 SignalStar Oligo-Antibody Pair #92797 (green), CD45 (Intracellular Domain) (D9M8I) & CO-0013-594 SignalStar Oligo-Antibody Pair #15734 (yellow), Phospho-SLP-76 (Ser376) (E3G9U) & CO-0018-647 SignalStar Oligo-Antibody Pair #83271 (red), CD16 (D1N9L) & CO-0031-750 SignalStar Oligo-Antibody Pair #48056 (cyan), and ProLong Gold Antifade Reagent with DAPI #8961 (blue). All fluorophores have been assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer.

Image Below: SignalStar™ multiplex immunohistochemical analysis of paraffin-embedded human gastric adenocarcinoma using CD163 (D6U1J) & CO-0022-594 SignalStar Oligo-Antibody Pair #43547 (magenta), Tox/Tox2 (E6I3Q) & CO-0016-488 SignalStar Oligo-Antibody Pair #31189 (green), α-Smooth Muscle Actin (D4K9N) & CO-0024-647 SignalStar Oligo-Antibody Pair #63902 (red), and Pan-Keratin (C11) & CO-0003-750 SignalStar Oligo-Antibody Pair #97227 (cyan). All fluorophores have been assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer.



Create Your Panel Today

Try the online panel builder to select and build panels specific to your research needs. We'll verify and ship your customized kit within 2 business days.

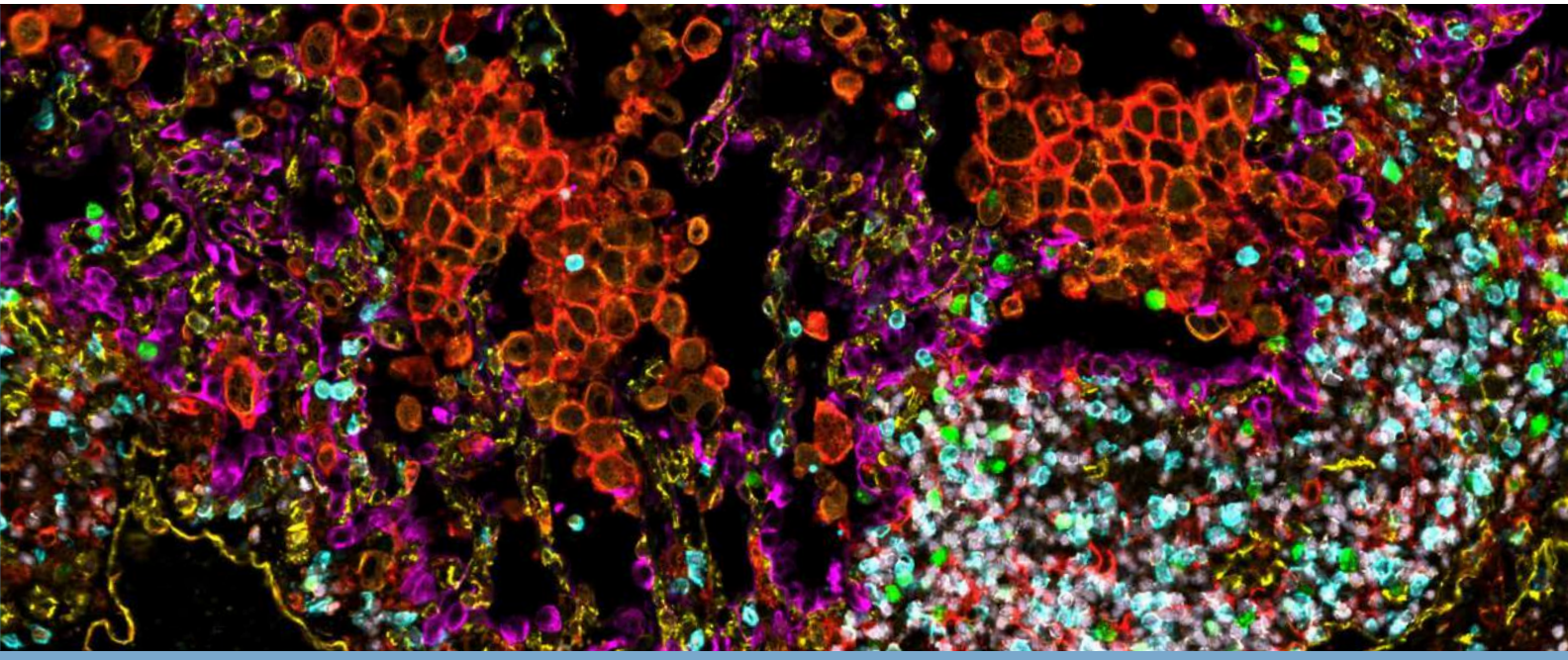


Image Above: SignalStar multiplex immunohistochemical analysis of paraffin-embedded human squamous cell lung carcinoma using Ki-67 (8D5) & CO-0014-488 SignalStar Oligo-Antibody Pair #89034 (green), Pan-Keratin (C11) & CO-0003-488 SignalStar Oligo-Antibody Pair #63566 (magenta), CD31 (PECAM-1) (89C2) & CO-0028-594 SignalStar Oligo-Antibody Pair #33420 (yellow), Vimentin (D21H3) & CO-0012-594 SignalStar Oligo-Antibody Pair #16471 (orange), CD11c (D3V1E) & CO-0017-647 SignalStar Oligo-Antibody Pair #96411 (red), TCF1/TCF7 (C63D9) & CO-0006-647 SignalStar Oligo-Antibody Pair #25502 (pink), CD8α (D8A8Y) & CO-0004-750 SignalStar Oligo-Antibody Pair #62750 (cyan), and CD3ε (D7A6E) & CO-0001-750 SignalStar Oligo-Antibody Pair #51754 (white). All fluorophores have been assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer.

Cell Signaling TECHNOLOGY®

1% FOR THE PLANET — MEMBER —

Local Sales Representative

Doing good science is important. So is just doing good.

Cell Signaling Technology (CST) is a *different* kind of life science company—one founded, owned, and led by active research scientists, with the highest standards of product and service quality, technological innovation, and scientific rigor for over 20 years. We consistently provide fellow scientists around the globe with best-in-class products and services to fuel their quests for discovery.

Helping researchers find new solutions is our main mission every day—but it's not our only mission. We're also dedicated to helping identify solutions to other problems facing our world. We believe that all businesses must be responsible and work in partnership with local communities, while seeking to minimize their environmental impact. That's why we joined 1% for the Planet as its first life Science member, and have committed to achieving net-zero emissions by 2029.

At CST, we believe in the power and promise of science to solve the challenges we face as a global community. We're a company of caring people driven by a devotion to facilitating good science—a company committed to doing the right thing for our Customers, our communities, and our planet.

Image Front Cover: **SignalStar™ technology enables visualization of 8 proteins simultaneously in formalin-fixed, paraffin-embedded (FFPE) tissue.** SignalStar multiplex immunohistochemical analysis of paraffin-embedded human gastric adenocarcinoma using Pan-Keratin (C11) & C0-0003-488 SignalStar Oligo-Antibody Pair #63566 (red), PD-L1 (E1L3N) & C0-0005-488 SignalStar Oligo-Antibody Pair #85646 (green), PD-1 (Intracellular Domain) (D4W2J) & C0-0008-594 SignalStar Oligo-Antibody Pair #35347 (yellow), Granzyme B (D6E9W) & C0-0009-594 SignalStar Oligo-Antibody Pair #15194 (orange), TIGIT (E5Y1W) & C0-0002-647 SignalStar Oligo-Antibody Pair #18288 (cyan), CD20 (E7B7T) & C0-0011-647 SignalStar Oligo-Antibody Pair #36775 (pink), CD3ε (D7A6E) & C0-0001-750 SignalStar Oligo-Antibody Pair #51754 (magenta), CD8α (D8A8Y) & C0-0004-750 SignalStar Oligo-Antibody Pair #62750 (white), and ProLong Gold Antifade Reagent with DAPI #8961 (blue). All fluorophores were assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer.

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