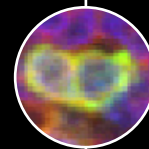
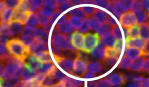
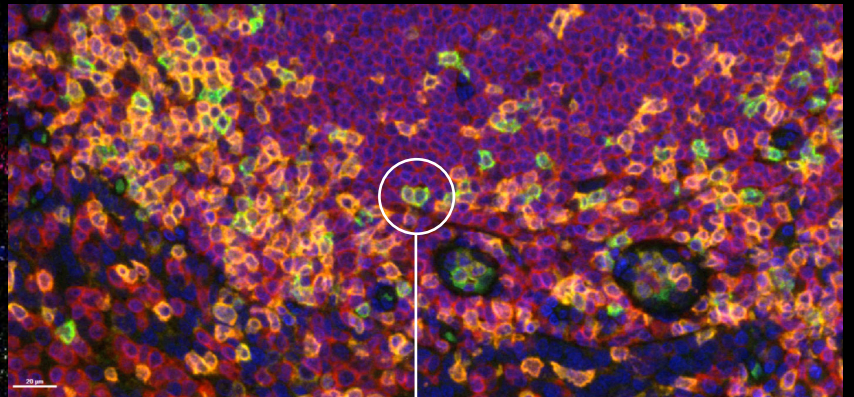
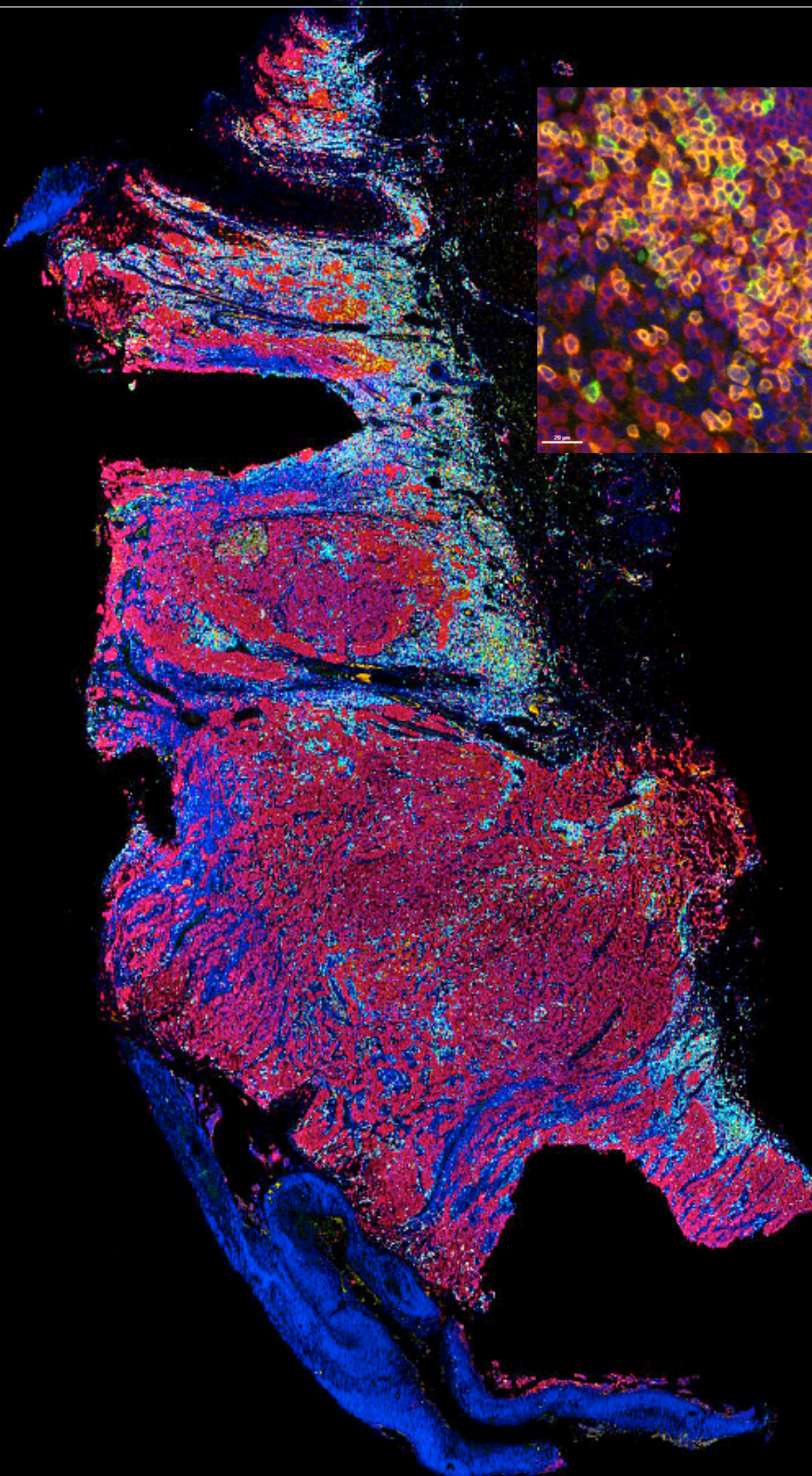
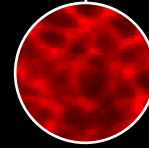
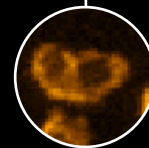
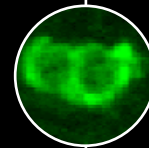
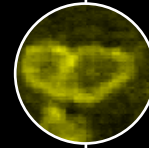


# Welcome To Opal

## Unlock the Power of Spatial Biology



**Detecting  
Multiple Markers  
of Interest on the  
Same Cell**

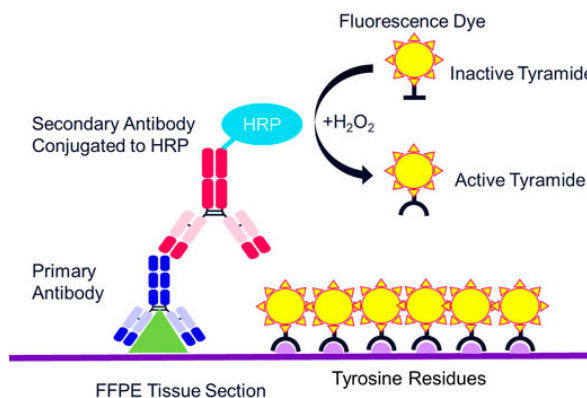




# Opal and TSA: Revolutionizing Immunohistochemistry

## What is TSA?

TSA, or Tyramide Signal Amplification, is a groundbreaking technology developed over two decades ago. It enhances immunofluorescence (IF) detection by utilizing horseradish peroxidase (HRP) to convert TSA molecules into free radicals that bind covalently to tyrosine residues near the targeted protein epitope (Figure 1). This open and versatile technology supports the use of any unlabeled primary antibody, including multiple antibodies raised in the same species. Labeling is performed sequentially with each color amplified individually, ensuring precise measurements with minimal bleed-through.



**Figure 1:** Mechanism of Tyramide Signal Amplification

## What is Opal-TSA?

Opal™-TSA represents the integration of Akoya's Opal fluorescent dyes with TSA technology, optimized for integration into multispectral IF platforms and facilitating up to 8-plex staining (9-colors including DAPI counterstain). Offered by Akoya Biosciences under the Opal trademark, Opal-TSA fluorophores have been carefully selected to provide optimum spectral separation across the visible wavelength range.

Opal-TSA is tailored for multiplex fluorescent immunohistochemistry on various sample types, including formalin-fixed, paraffin-embedded (FFPE) tissues or cells, tissue microarrays and frozen sections. This innovative technique can be performed manually or through automated processes and employs reactive fluorophores that covalently attach to the target epitope, allowing for sequential detection of multiple targets without antibody cross-reactivity. After each detection step, antibodies are removed in a way that preserves the fluorescence signal, enabling the development of multiplexed assays that deliver balanced, quantitative signals for both rare and abundant targets.

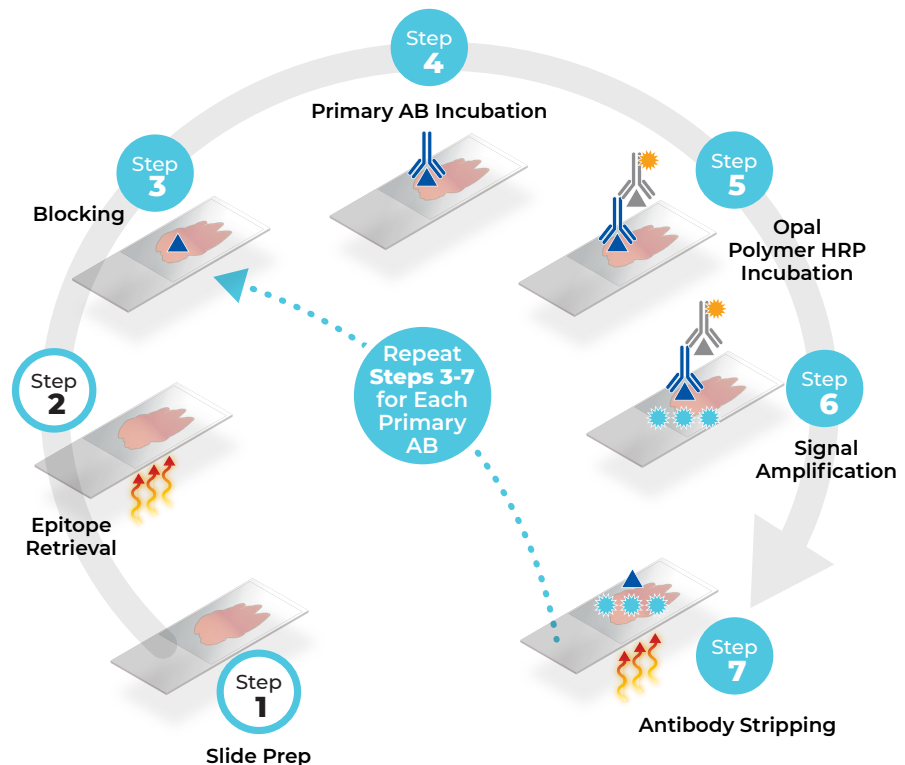
Opal-TSA method is exceptionally photostable, allowing for the archival and re-analysis of slides up to a year after staining with minimal signal degradation. Slides stained with this method can be imaged in a single scan using the advanced multispectral imagers from the Akoya portfolio, including the Phenolmager™ HT and Fusion.



# Opal Enables You To...

- Increase multiplexing for diverse biomarker detection strategies and add signal amplification to nearly any immunoassay
- Identify multiple cell phenotypes while preserving spatial and morphological context, which is lost with bulk measurements and flow cytometry
- Extract more information from precious and scarce samples while reducing antibody consumption
- Utilize the best primary antibodies, irrespective of species origin, without cross-reactivity concerns
- Improve sensitivity by 10 to 100-fold and achieve superior resolution with low background
- Attain a higher dynamic range (up to 4 logs) compared to chromogenic methods (up to 1 log)

## Iterative Staining Diagram for Opal Multiplex IHC Assays



Following the steps of baking & dewaxing (Step 1) and epitope retrieval (Step 2), slides are blocked (Step 3) and then stained with the first primary antibody (Step 4). A single antibody is revealed at a time, beginning with the incubation of a secondary antibody conjugated to HRP. Signal amplification is then performed using Opal-TSA chemistry. Once complete, the antibodies are stripped away while preserving the fluorescence signal. The process (steps 3-7) is repeated for each antibody, labeling the markers with different Opal dyes.

Contact us today to learn more about in-person and virtual Akoya Academy sessions!

## OPAL MANUAL MULTIPLEX IHC DETECTION KITS

Opal™ kits come complete with all your necessary secondary antibodies and detection reagents for manual multiplex IHC staining.

Name	Size	Part #
Opal 6-Plex Manual Detection Kit - for Whole Slide Imaging (* for use with HT 2.0 onboard spectral unmixing)	Up to 50 Slides	NEL861001KT
Opal 6-Plex Manual Detection Kit	Up to 50 Slides	NEL811001KT
Opal 3-Plex Manual Detection Kit	Up to 50 Slides	NEL810001KT
Opal 3-Plex anti-Rabbit Manual Detection Kit	Up to 50 Slides	NEL840001KT
Opal Anti-Rb HRP Kit	Up to 50 Slides	ARR1001KT
Opal Anti-Ms + Rb HRP Kit	Up to 50 Slides	ARH3001KT

**LEARN MORE:** [www.akoyabio.com/phenoimager/assays/](http://www.akoyabio.com/phenoimager/assays/)

## OPAL AUTOMATION MULTIPLEX IHC DETECTION KITS

Opal™ kits come complete with all your necessary secondary antibodies and detection reagents for use with the Leica® BOND RX™ system.

Name	Size	Part #
Opal 6-Plex Detection Kit - for Whole Slide Imaging (* for use with HT 2.0 onboard spectral unmixing)	Up to 50 Slides	NEL871001KT
Opal 6-Plex Detection Kit	Up to 50 Slides	NEL821001KT
Opal 3-Plex Detection Kit	Up to 50 Slides	NEL820001KT
Opal 3-Plex anti-Rabbit Detection Kit	Up to 50 Slides	NEL830001KT

**LEARN MORE:** [www.akoyabio.com/phenoimager/assays/](http://www.akoyabio.com/phenoimager/assays/)

## OPAL STAINING ESSENTIALS

Additional reagents optimized for use with Opal-TSA staining methods. Includes antibody blocking buffer, pH 6 or pH 9 antigen retrieval buffers and amplification diluents for automated and manual workflows.

Name	Size	Part #
1X Antibody Diluent / Block	100 mL	ARD1001EA
1X Plus Automation Amplification Diluent	50 mL	FP1609
1X Plus Manual Amplification Diluent	50 mL	FP1498
10X AR6 Buffer	250 mL	AR600250ML
10X AR9 Buffer	250 mL	AR900250ML

**LEARN MORE:** [www.akoyabio.com/phenoimager/assays/](http://www.akoyabio.com/phenoimager/assays/)

## OPAL FLUOROPHORE REAGENT PACKS

Standalone Opal Fluorophore reagent packs allow you to pick and choose your panel's biomarker quantity and design.

Name	Size	Part #
Opal 480 Reagent Pack	Up to 50 Slides	FP1500001KT
Opal 520 Reagent Pack	Up to 50 Slides	FP1487001KT
Opal 540 Reagent Pack	Up to 50 Slides	FP1494001KT
Opal 570 Reagent Pack	Up to 50 Slides	FP1488001KT
Opal 620 Reagent Pack	Up to 50 Slides	FP1495001KT
Opal 650 Reagent Pack	Up to 50 Slides	FP1496001KT
Opal 690 Reagent Pack	Up to 50 Slides	FP1497001KT
Opal 780 Reagent Pack	Up to 50 Slides	FP1501001KT
10X Spectral DAPI	Up to 50 Slides	FP1490

**LEARN MORE:** [www.akoyabio.com/phenoimager/assays/opal-fluorophore-reagent-packs/](http://www.akoyabio.com/phenoimager/assays/opal-fluorophore-reagent-packs/)

Contact us today to learn more about in-person and virtual Akoya Academy sessions!

# PhenoCode Signature

## Ready-to-use Human Protein Panels

Explore the forefront of biomarker development, immunotherapy response, and next-generation therapeutic strategies with PhenoCode™ Signature panels. These panels are designed to offer the relevant content and adaptability required to effectively navigate the rapidly evolving combination therapy landscape.

Leveraging **molecular barcoding technology** from Akoya's PhenoCycler™ platform, formerly known as CODEX, combined with the enhanced sensitivity of **Opal-TSA technology**, PhenoCode Signature panels allow for a detailed analysis of core immune phenotypes of the tumor microenvironment. Additionally, these panels provide the versatility to seamlessly incorporate an extra marker into a foundational 5-plex panel, ensuring that your research stays at the cutting edge of scientific discovery.

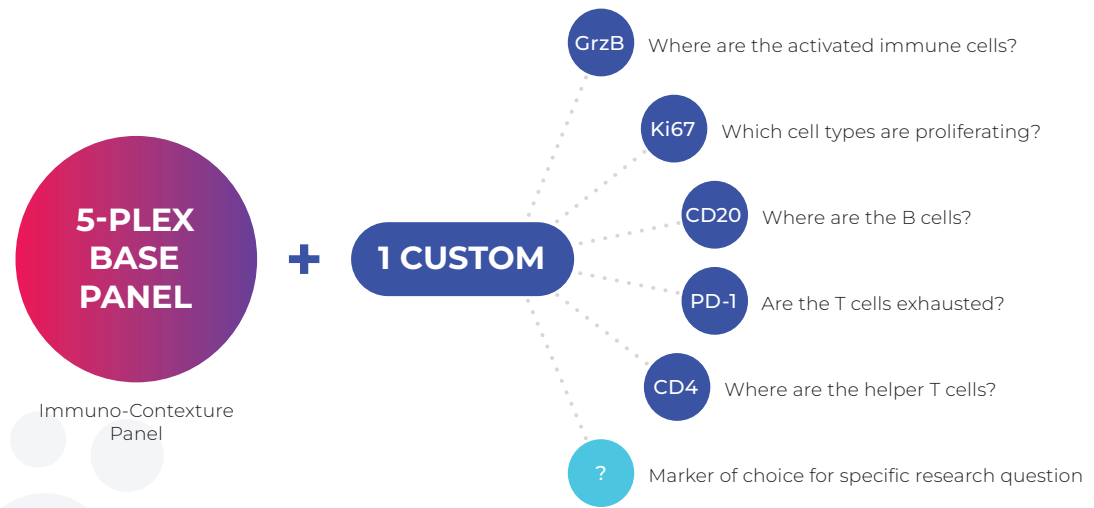
## Start with asking the right questions

Choose from our menu of complementary 5-plex base panels to map the tumor micro-environment.

<p><b>Immuno-Contexture Panel</b></p> <p>CD8 CD68 PD-L1 FoxP3 PanCK</p> <p>Is the tumor "hot" or "cold"?</p>	<p><b>Immune Profile Panel</b></p> <p>CD8 CD68 CD3 CD20 PanCK</p> <p>Where are the immune cells in the TME?</p>	<p><b>Activated TIL Status Panel</b></p> <p>CD8 CD3 Ki67 GrzB PanCK</p> <p>Are the tumor cells proliferating or lymphocytes activated?</p>	<p><b>M1/M2 Polarization Panel</b></p> <p>CD8 CD68 CD163 PD-1 PD-L1</p> <p>Where are the TAMs in relation to the tumor margin?</p>	<p><b>T Cell Status Panel</b></p> <p>CD8 CD4 FoxP3 CD20 PD-1</p> <p>Where are the exhausted and regulatory T cells?</p>
------------------------------------------------------------------------------------------------------------------------------	---------------------------------------------------------------------------------------------------------------------------------	------------------------------------------------------------------------------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------

## Flexibility to answer more questions quickly

Easy integration of one additional marker to a 5-plex panel. Analyze additional cell phenotypes or address your specific research question with your own marker of choice.

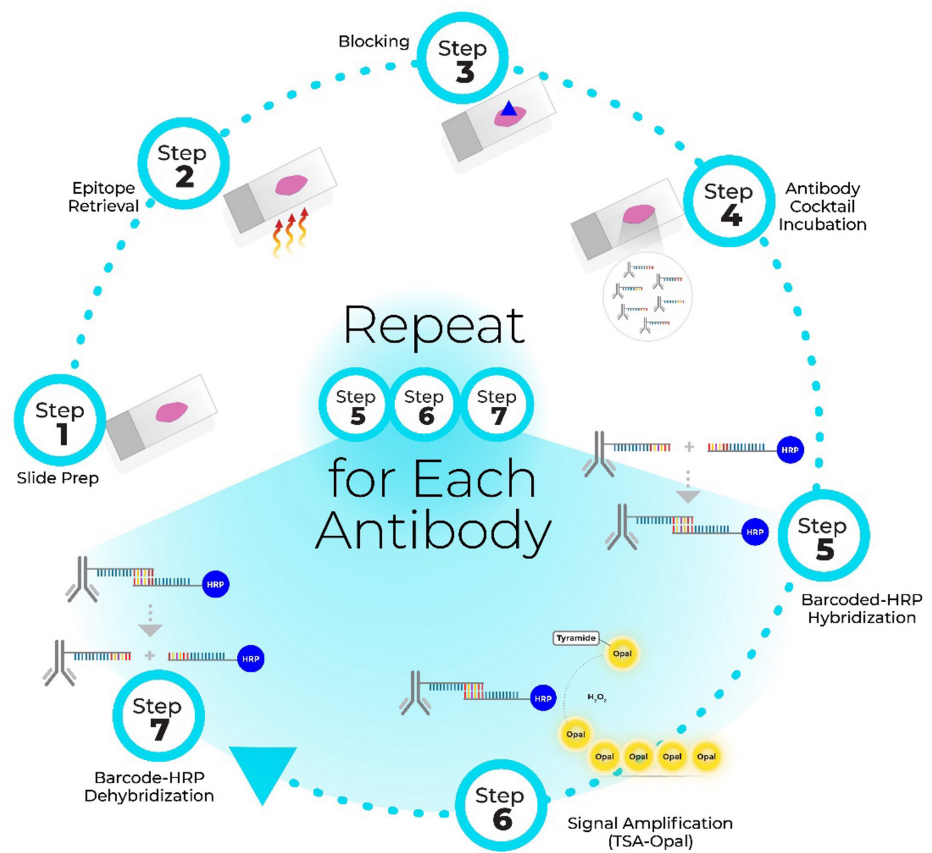


Contact us today to learn more about in-person and virtual Akoya Academy sessions!

# PhenoCode Signature Enables You To...

- **RELEVANT** – Answer the most pertinent questions to interrogate the TME
- **FLEXIBLE** – Answer novel questions by adding your marker of choice
- **FAST** – Speed up spatial signature development by 3X
- **SCALABLE** – Seamlessly translate discoveries into predictive biomarkers with the Phenolmager® solution

## Iterative Staining Method for PhenoCode Signature Assays



Following the steps of baking and dewaxing (Step 1) and epitope retrieval (Step 2), slides are stained with a primary antibody cocktail (Step 4), in which antibodies have been conjugated to unique oligo-based barcodes. A single antibody is revealed at a time, beginning with the hybridization of a complementary barcode conjugated to HRP (Step 5). Signal amplification is then performed using Opal-TSA chemistry (Step 6). Once complete, the HRP conjugate is dehybridized (Step 7). The process (steps 5-7) is repeated for each antibody, labeling the markers with different Opal dyes.

Contact us today to learn more about in-person and virtual Akoya Academy sessions!

## Key Advantages of PhenoCode Signature

- **Workflow Efficiencies** – Single step primary antibody incubation
- **No Secondary Antibody** – Highly specific barcode-based hybridization
- **Strong Signal Intensities** – Opal-tyramide signal amplification

### PHENOCODE SIGNATURE HUMAN PROTEIN PANELS

PhenoCode Signature panels are validated for the PhenoImager platform, and each panel contains 5 Primary antibodies, 5 PhenoCode Signature Detectors, 5 Opal dyes and Spectral DAPI, and the necessary diluents and blockers.

Name	Size	Part #
PhenoCode Signature Immuno-Contexture Human Protein Panel	Up to 20 Slides	PCSP001
PhenoCode Signature Immune Profile Human Protein Panel	Up to 20 Slides	PCSP002
PhenoCode Signature Activated TIL Status Human Protein Panel	Up to 20 Slides	PCSP003
PhenoCode Signature M1/M2 Polarization Human Protein Panel	Up to 20 Slides	PCSP004
PhenoCode Signature T Cell Status Human Protein Panel	Up to 20 Slides	PCSP005

**LEARN MORE:** [www.akoyabio.com/phenoimager/assays/](http://www.akoyabio.com/phenoimager/assays/)

### PHENOCODE SIGNATURE ANTIBODIES, KITS AND REAGENTS

PhenoCode Signature antibodies, kits and reagents have been optimized and validated for the PhenoImager systems and provide users the flexibility for panel customization. The antibodies listed below come pre-barcoded and can be seamlessly integrated into the PhenoCode Signature panels mentioned in the table above. To add a 6th antibody to any PhenoCode Signature panel, the Opal 520 reagent pack is required. The Antibody Conjugation Kit for PhenoCode Signature can be used to add a preferred marker of choice to any of the PhenoCode Signature in the table above. The Staining Kit for PhenoCode Signature can be used to create and optimize a user customized panel.

Name	Size	Part #
Anti-Hu Pan-Cytokeratin (AKYP0053)-BX066 for PhenoCode Signature	Up to 20 Slides	S6501000
Anti-Hu CD8 (AKYP0028)-BX026 for PhenoCode Signature	Up to 20 Slides	S6501001
Anti-Hu CD4 (AKYP0048)-BX003 for PhenoCode Signature	Up to 20 Slides	S6501002
Anti-Hu CD3e (AKYP0125)-BX080 for PhenoCode Signature	Up to 20 Slides	S6501003
Anti-Hu CD68 (AKYP0050)-BX015 for PhenoCode Signature	Up to 20 Slides	S6501004
Anti-Hu CD163 (AKYP0114)-BX069 for PhenoCode Signature	Up to 20 Slides	S6501005
Anti-Hu Ki67 (AKYP0126)-BX047 for PhenoCode Signature	Up to 20 Slides	S6501006
Anti-Hu FoxP3 (AKYP0102)-BX031 for PhenoCode Signature	Up to 20 Slides	S6501007
Anti-Hu CD20 (AKYP0049)-BX064 for PhenoCode Signature	Up to 20 Slides	S6501008
Anti-Hu Granzyme B (AKYP0086)-BX041 for PhenoCode Signature	Up to 20 Slides	S6501009
Anti-Hu PD-1 (AKYP0127)-BX046 for PhenoCode Signature	Up to 20 Slides	S6501010
Anti-Hu PD-L1 (AKYP0103)-BX067 for PhenoCode Signature	Up to 20 Slides	S6501011
Anti-Hu CD45RO (AKYP0059)-BX017 for PhenoCode Signature	Up to 20 Slides	S6501012
Anti-Hu SMA (AKYP0081)-BX013 for PhenoCode Signature	Up to 20 Slides	S6501013
Antibody Conjugation Kit for PhenoCode Signature	Up to 20 Slides	PCSP0200
Opal 520 Reagent Pack	Up to 50 Slides	FP1487001KT
Staining Kit for PhenoCode Signature	Up to 20 Slides	PCSP0100

Require  
technical  
assistance?  
Get help at  
[support@  
akoyabio.com](mailto:support@akoyabio.com)

## Additional Resources

Please visit [www.akoyabio.com/support](http://www.akoyabio.com/support) for additional resources, including FAQs and publications.

For more information on the Opal and PhenoCode Signature regents, methodology and workflow, please visit our website [www.akoyabio.com/phenoimager/assays/](http://www.akoyabio.com/phenoimager/assays/).

For information on publication using Opals, PhenoCode Signature or PhenoImager Instruments, please visit [www.akoyabio.com/publication/](http://www.akoyabio.com/publication/).

Reach out to us at [customercare@akoyabio.com](mailto:customercare@akoyabio.com) with any questions or to request a quote today.

To learn more visit [AKOYABIO.COM](http://AKOYABIO.COM) or email us at [INFO@AKOYABIO.COM](mailto:INFO@AKOYABIO.COM)

For Research Use Only. Not for use in diagnostic procedures.

© 2024 Akoya Biosciences, Inc. All rights reserved. LEICA is a registered trademark of Leica Microsystems IR GmbH. BOND and BOND RX are trademarks of Leica Biosystems Melbourne Pty. Ltd. All trademarks are the property of Akoya Biosciences unless otherwise specified.

DN-00062 Rev B