

# PhenoCode Signature Immune Profile Human Protein Panel

CATALOG #PCSP002

## PRODUCT INFORMATION

See [Page 3](#) for detailed information.

### QUANTITY

Up to 20 slides

### CONTENTS

PhenoCode Signature Antibodies

- CD8
- CD68
- CD3e
- CD20
- Pan-Cytokeratin

PhenoCode Signature Detectors

Opal® dyes + DAPI

- Opal 480
- Opal 570
- Opal 620
- Opal 690
- Opal 780 + TSA-DIG

Blockers , Buffers, Diluents

- 1X Plus Automation Amplification Diluent
- PhenoCode Signature Diluent
- 1X Antibody Diluent/Block
- PhenoCode Signature Blocker 1
- PhenoCode Signature Blocker 5
- N Blocker
- J Blocker
- S Blocker

### STORAGE & STABILITY

Storage: See [Page 3](#)

Stability: Refer to individual components

### SPECIES REACTIVITY

Human

### TISSUE TYPE

FFPE

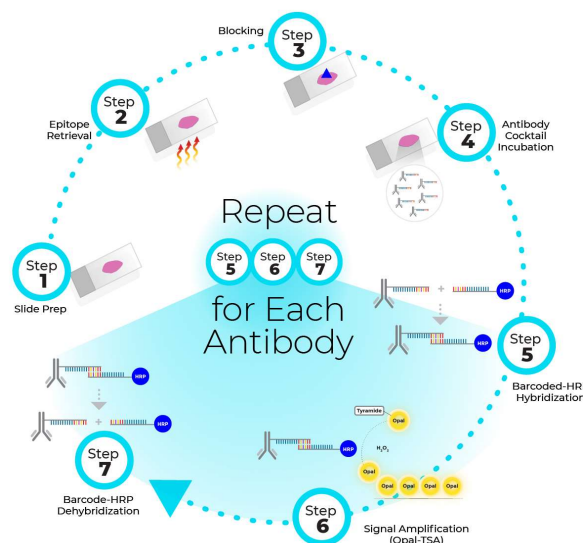
### SYSTEMS COMPATIBILITY

Staining: Leica® BOND RX™

Imaging: Phenolmager® systems

PhenoCode™ Signature Immune Profile Human Protein Panel allows for the rapid profiling of the major subtypes of immune cells in the tumor microenvironment. Detect up to 6 key biomarkers using a fixed 5-plex base panel (CD8, CD68, CD3e, CD20, Pan-Cytokeratin) with the flexibility to add 1 additional marker (+1 antibody) to map additional phenotypes and answer more questions.

## PhenoCode Signature Workflow



## PhenoCode Signature Technology

Benefit	PhenoCode Signature Technology Feature	
	Antibodies	Detection
Easy optimization across various tissues	Provided as concentrated stock	Antibody and Opal dye assignments fixed
Reliable chemistry	Well-documented protein barcoding used in 1-step antibody cocktail for high specificity binding	Highly-published Opal-TSA signal amplification for subcellular sensitivity

## Suggested +1 Antibodies for Additional Phenotyping

Catalog #	Biomarker	Phenotype/Question Addressed
S6501002	CD4	Where are the Helper T cells?
S6501010	PD-1	Are the T cells exhausted?
S6501011	PD-L1	Are tumor cells suppressing immune response?
S6501006	Ki67	Are tumor cells proliferating?
S6501007	FoxP3	Where are the Regulatory T cells?
PCSP0200*	VARIABLE	What matters the most?

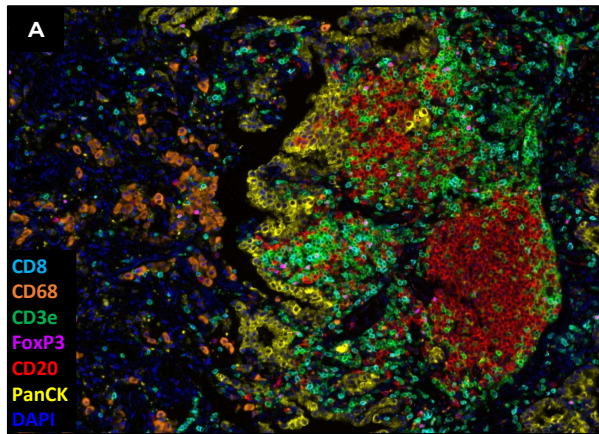
# PhenoCode Signature Immune Profile Human Protein Panel

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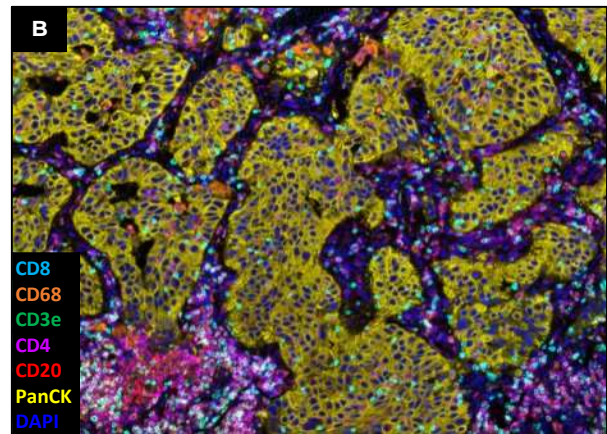
Human FFPE lung cancer and tonsil tissues were stained with PhenoCode Signature Immune Profile Human Protein Panel using the setup detailed in the table below.

Antibody	Cellular Expression	Expected Localization	Clone ID	Recommended Antibody Dilution	Opal Dye	Detection Order
Pan-Cytokeratin	Epithelial cells	Cytoplasm	AKYP0053	1:4000	Opal 690	1
CD3e	T cells	Membrane	AKYP0125	1:12000	Opal 570	2
CD68	Pan-Macrophage	Membrane	AKYP0050	1:8000	Opal 780	3
+1 VARIABLE	VARIABLE	VARIABLE	VARIABLE	VARIABLE	Opal 520 sold separately	4
CD20	B cells	Membrane	AKYP0049	1:800	Opal 620	5
CD8	Cytotoxic T cells, some NK cells	Membrane	AKYP0028	1:2000	Opal 480	6

## Human FFPE Lung Cancer

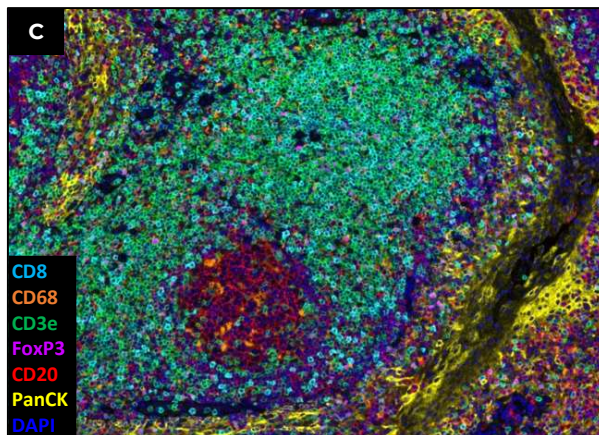


PhenoCode Signature Immune Profile panel + FoxP3 as the variable antibody

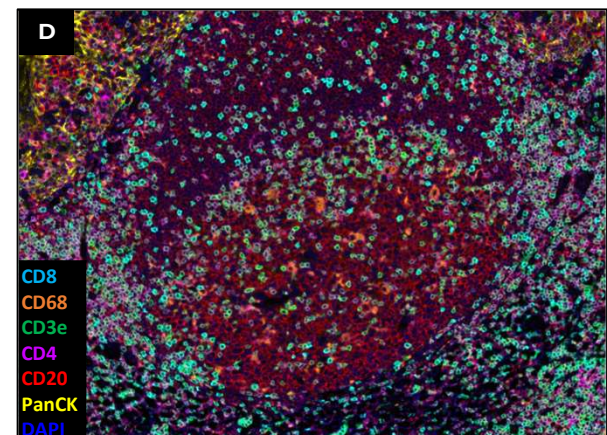


PhenoCode Signature Immune Profile panel + CD4 as the variable antibody

## Human FFPE Tonsil



PhenoCode Signature Immune Profile panel + FoxP3 as the variable antibody



PhenoCode Signature Immune Profile panel + CD4 as the variable antibody

Human FFPE lung cancer section was stained with PhenoCode Signature Immune Profile Human Protein Panel according to the setup provided in the table. Slides were imaged using the Phenolmager HT. Images shown are at 20X and detail the +1 variable antibody. **A.** Anti-FoxP3 was added to the panel and diluted at 1:100. **B.** Anti-CD4 was added to the panel and diluted at 1:800. **C and D.** Identical assays were run on human FFPE tonsil tissue and images are displayed in the same manner as in figures A and B.

# PhenoCode Signature Immune Profile Human Protein Panel

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## SUPPLIED MATERIALS

The PhenoCode Signature Human Protein Panel kit contains antibodies, buffers, and reagents to perform multiplex immunofluorescence staining of 5 biomarkers in up to 20 slides of human formalin-fixed, paraffin-embedded (FFPE) tissue. Blockers, buffers, and diluents are provided in excess to accommodate 1 additional antibody of interest to create a 6-plex panel. Prepare library slides every 6 months and an autofluorescence slide for each tissue type per staining run to ensure accurate spectral unmixing for downstream analysis. Purchase of additional reagents may be necessary to create library and autofluorescence slides.

**IMPORTANT** Before beginning this assay, select one additional biomarker of interest. Choose from Akoya's menu of PhenoCode Signature antibodies (S650XXXX), barcode your own marker using the Antibody Conjugation Kit for PhenoCode Signature (PCSP0200) or use Akoya's antibody [barcoding service](#). To detect the additional marker of interest, purchase Opal 520 Reagent Pack (FP1487001KT). Image using an Akoya Phenolmager system.

Reagents stain up to 20 slides (max 4 batches of 5 slides each) on the Leica BOND RX running software version 6 or above.

Component #	Component Name	Units	Storage Notes	Storage Temp	Shipment Temp
<b>PhenoCode Signature Detectors</b>					
PCSD080	HRP-HX080 PhenoCode Signature Detector	1	Do Not Exceed 5 Freeze-Thaw Cycles	-20°C	-20°C
PCSD026	HRP-HX026 PhenoCode Signature Detector	1			
PCSD015	HRP-HX015 PhenoCode Signature Detector	1			
PCSD064	HRP-HX064 PhenoCode Signature Detector	1			
PCSD066	HRP-HX066 PhenoCode Signature Detector	1			
<b>Blockers &amp; Buffers</b>					
200040	PhenoCode Signature Blocker 5	2	N/A	-20°C	-20°C
200039	PhenoCode Signature Blocker 1	1			
232111	S Blocker	1			
232108	N Blocker	1	N/A	4°C	4°C
232110	J Blocker	1			
200035	1X Antibody Diluent / Block	1			
FP1609	1X Plus Automation Amplification Diluent	1			
PCSA1001	PhenoCode Signature Diluent	1			
<b>PhenoCode Signature Antibodies</b>					
240227	Anti-Hu CD3e (AKYP0125)-BX080	1	Do Not Freeze	4°C	4°C
232151S	Anti-Hu CD8 (AKYP0028)-BX026	1			
232176	Anti-Hu CD68 (AKYP0050)-BX015	1			
240230	Anti-Hu CD20 (AKYP0049)-BX064	1			
240229	Anti-Hu Pan-Cytokeratin (AKYP0053)-BX066	1			
<b>Opal Dyes &amp; Staining Reagents</b>					
OP-001000	Opal 480 Reagent	1	*Opal dyes ship at 4 °C. Store in the dark at -20 °C.  Protect from light.  Once reconstituted, store at 4 °C in the dark.	-20°C	4°C
OP-001003	Opal 570 Reagent	2			
OP-001004	Opal 620 Reagent	1			
OP-001006	Opal 690 Reagent	1			
OP-001008	Opal 780 Reagent	1			
OP-001007	Opal TSA-DIG Reagent	2			
FP1490	10X Spectral DAPI	1	Protect from light.	4°C	4°C
200045	DMSO	1		Ambient/RT	4°C
*Store reconstituted Opal 780 at 4 °C in ddH <sub>2</sub> O for up to 30 days and store all other reconstituted Opals at 4 °C in DMSO for up to 90 days.					

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## MATERIALS NOT PROVIDED

Required to prepare 20 slides:

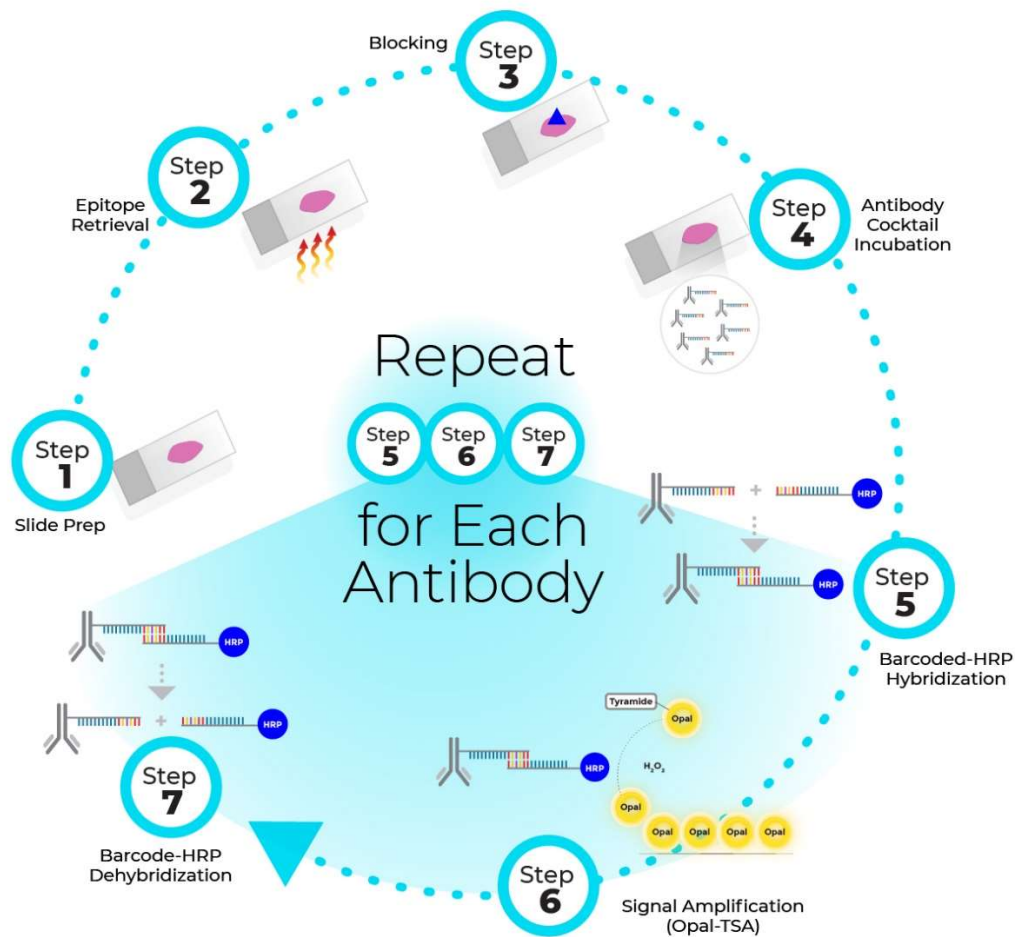
	Item	Recommended Vendor &/or Minimum Volume	Recommended Vendor Part Number
Laboratory Materials	30% Hydrogen Peroxide (H <sub>2</sub> O <sub>2</sub> )	VWR, 1 mL	BDH7690-1
	100% Reagent Grade Alcohol	VWR, 3 mL	89370-084
	16% Paraformaldehyde (PFA)	Electron Microscopy Sciences, 2 mL	15710
	1X PBS, pH 7.4	Customer Choice, 40 mL	N/A
	Double-distilled water (ddH <sub>2</sub> O)	Customer Choice, approx. 255 mL	N/A
	PhenoCode Signature Antibody	Akoya Biosciences	S650XXXX
	Opal 520 Reagent Pack	Akoya Biosciences	FPI487001KT
	Mounting media: fluorescent-compatible, hard-set, DAPI-free	Thermo Fisher Scientific: ProLong™ Diamond Antifade Mountant	P36965
	Glass coverslips, #1.5	Customer Choice	N/A
	Vortex	Customer Choice	N/A
	Tabletop Centrifuge	Customer Choice	N/A
BOND RX Materials	Bond Epitope Retrieval Solution 1	Leica Biosystems	AR9961
	Bond Epitope Retrieval Solution 2		AR9640
	Bond Research Detection System 2		DS9777
	Bond Slide Label & Print Ribbon Kit		Several Options
	Bond Universal Covertiles		S21.4611
	Bond Titration Kit		OPT9049
	Wash Solution 10X Concentrate		AR9590
	Dewax Solution		AR9222



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## EXPERIMENT OVERVIEW



**FIGURE 1. PhenoCode Signature Workflow.** After dewaxing, epitope retrieval, and blocking, tissue sections are stained with the PhenoCode Signature panel antibody cocktail. Antibodies are conjugated to unique oligo barcodes and applied to tissue in a single cocktail incubation step (Steps 1-4). Individual antibodies are detected one at a time, beginning with the hybridization of a complementary oligo conjugated to horseradish peroxidase (HRP). Signal amplification is performed using Opal chemistry. The detection process is repeated for each antibody until all Opal dyes have been deposited onto the tissue (Steps 5-7).

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## SLIDE PREPARATION

Bake FFPE slides at 65° C vertically in an oven for 3 hours.

**NOTE** When using tissue microarrays (TMAs), rotating slides 90 degrees each hour helps ensure even baking and is recommended to prevent pooling of wax on edges of tissue.

Deparaffinization, dehydration, antigen retrieval, and wash steps are completed on the Leica BOND RX autostainer using the Dewax and HIER preparation protocols defined in the BOND RX Protocol Setup & Staining section.

## REAGENT PREPARATION

Before beginning reagent preparation, remove frozen reagents from storage and thaw at room temperature (RT). Recommended antibody dilutions in Table 1 provide a starting point for panel optimization, as individual tissue properties and inherent variance across tissue types can influence the amount of each antibody required for detection.

TABLE 1. Panel Setup & Recommended Antibody Dilutions

Detection Order	Antibody	Recommended Antibody Dilution	PhenoCode Signature Detector	PhenoCode Signature Detector Dilution	Opal Dye	Opal Dye Dilution
1	PanCK	1:4000	HRP-HX066	1:100	Opal 690	1:150
2	CD3e	1:12000	HRP-HX080	1:100	Opal 570	1:100
3	CD68	1:8000	HRP-HX015	1:100	TSA-DIG, Opal 780*	1:100, 1:25
4	VARIABLE	VARIABLE	VARIABLE	1:100	Opal 520	1:150
5	CD20	1:800	HRP-HX064	1:100	Opal 620	1:150
6	CD8	1:2000	HRP-HX026	1:100	Opal 480	1:150

\*Opal 780 is dispensed last and accompanies Opal TSA-DIG.

## PFA Preparation

Prepare fresh solution for each experiment.

1. Prepare 4% PFA by diluting 16% PFA stock solution with 1X PBS in a 6 mL Bond Titration vial under a fume hood.
2. Determine the final volume of PFA according to the guidelines in Table 2. Volumes in the table are based on total number of slides run. BOND RX dead volumes are accounted for in the table.
3. Label and film seal or cap the titration vial. Set aside at RT.

TABLE 2. Guidelines for Preparing PFA

Reagent	Volume per Number of Slides (µL)			
	≤4 Slides	≤11 Slides	≤18 Slides	≤20 Slides
16% PFA	250	500	750	1000
1X PBS	750	1500	2250	3000
Total Volume	1000	2000	3000	4000

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## Alcohol Solution Preparation

Prepare fresh solution for each experiment. Determine the volume of Alcohol Solution needed using Table 3; consider total number of slides to be run, including experimental, control, and autofluorescence slides.

- Prepare 3% H<sub>2</sub>O<sub>2</sub> in 50% alcohol using a 6 mL Bond Titration vial under a fume hood using the guidelines in Table 3. BOND RX dead volumes are accounted for in the table.
- Label and film seal or cap the titration vial. Set aside at RT.

TABLE 3. Guidelines for Preparing Alcohol Solution

Reagent	Volume per Number of Slides (µL)			
	≤4 Slides	≤11 Slides	≤18 Slides	≤20 Slides
30% H <sub>2</sub> O <sub>2</sub>	100	200	300	400
ddH <sub>2</sub> O	400	800	1200	1600
100% Reagent Grade Alcohol	500	1000	1500	2000
Total Volume	1000	2000	3000	4000

## Antibody Cocktail Preparation

- Determine volumes of Antibody Cocktail and Antibody Cocktail Diluent using the Reagents Calculation Tables.

**NOTE** The Reagents Calculation Tables take into account BOND RX dead volumes.

- Allow S Blocker and PhenoCode Signature Blocker 1 to thaw and equilibrate to RT.
- Retrieve N Blocker, J Blocker, and 1X Antibody Diluent/Block from 4°C storage.
- Vortex the following blocking reagents (**do not vortex >3 seconds**) and spin down for 5-10 seconds:
  - N Blocker
  - J Blocker
  - S Blocker
  - PhenoCode Signature Blocker 1
- Prepare the Antibody Cocktail Diluent by adding each of the above 4 blocking buffers to 1X Antibody Diluent/Block according to the volumes indicated in the Reagent Calculation Table based on number of slides desired. Vortex briefly (**do not vortex >3 seconds**) or gently mix by inverting several times.
- Gently mix each tube of concentrated antibody stock by inverting several times or vortex (**do not vortex >3 seconds**), then spin down for 5-10 seconds.
- Prepare the Antibody Cocktail in a Bond Titration vial by adding each antibody to the Antibody Cocktail Diluent according to the Reagents Calculation Table. If the volume of concentrated antibody stock is less than 1 µL, use a 2-step dilution process. For antibodies that require a two-step dilution, use Antibody Cocktail Diluent for both dilution steps. For the first step use 1 µL antibody stock in 99 µL Antibody Cocktail Diluent and for the second step refer to the Reagents Calculation Table for guidance.
- Vortex briefly (**do not vortex >3 seconds**) or gently mix by inverting several times. Label and film seal or cap the titration vial. Set aside at RT.

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## Detector Preparation

- Allow PhenoCode Signature Blocker 5, PhenoCode Signature Diluent, and PhenoCode Signature Detectors (ex. HRP-HX026, HRP-HX066, etc.) to thaw and equilibrate to RT.

**NOTE** Do not exceed 5 freeze-thaw cycles of PhenoCode Signature Detectors.

- Gently mix each PhenoCode Signature Detector by pipetting up and down (**do not vortex**), then spin down for 5-10 seconds.
- Prepare Detector Diluent according to the Reagents Calculation Tables using PhenoCode Signature Blocker 5 and PhenoCode Signature Diluent.
- Use 6 mL Bond Titration vials to prepare each barcode solution at a 1:100 dilution according to volumes in the Reagents Calculation Tables by combining PhenoCode Signature Detector with the Detector Diluent prepared above.
- Gently mix by pipetting up and down (**do not vortex**). Label and film seal or cap the titration vials. Set aside at RT.

## Opal Dye Preparation

**NOTE** Opal dyes, DMSO, and Spectral DAPI are light sensitive. Take precautions to protect from light exposure.

- Allow powdered Opal dyes to thaw and equilibrate to RT. If using Opal dyes that have been reconstituted, vortex (**do not vortex >10 seconds**) and spin down each of the dyes for 5-10 seconds, then skip to Step 22.
- Once the Opal dyes and DMSO have reached RT, spin down the powdered Opal dyes and reconstitute according to the guidelines in Table 4.

TABLE 4. Preparation of Opal Dyes

Reagent	Opal 480	Opal 520	Opal 570	Opal 620	Opal 690	Opal TSA-DIG	Opal 780
<b>Opal Dye Reconstitution</b>							
Diluent Volume (µL)	75	75	75	75	75	75	300
Diluent	DMSO						ddH <sub>2</sub> O
<b>Opal Dye Working Solution</b>							
Dilution Factor	1:150	1:150	1:100	1:150	1:150	1:100	1:25
Diluent	1X Plus Automation Amplification Diluent						1X Antibody Diluent/Block

- Vortex all reconstituted Opal dyes (**do not vortex >10 seconds**) and spin down for 5-10 seconds to create Opal dye stock solutions. Wait 15 minutes for the Opal to dissolve, then repeat one time.
- To create working solutions of each Opal dye (except Opal 780), dilute the Opal dye stock solutions in 1X Plus Automation Amplification Diluent according to the Reagents Calculation Tables. If preparing 17 slides or more for staining, split the total volume of each Opal dye working solution between 2 Bond 6 mL Titration vials to avoid exceeding the maximum volume of the vial. For additional details see the 20 slide Reagents Calculation Table. Dead volumes have been taken into account.
- To create Opal 780 working solution, dilute the Opal 780 stock solution in 1X Antibody Diluent/Block according to the Reagent Calculation Tables.
- Vortex (**do not vortex >10 seconds**) and film seal or cap the titration vials. Label and set aside at RT protected from light.



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## Spectral DAPI Preparation

25. Add 3 drops of 10X Spectral DAPI solution per 1 mL of 1X PBS. Use the Reagents Calculation Tables to determine the final volume needed. If preparing 17 slides or more for staining, split the total volume of DAPI between 2 Bond 6 mL Titration vials to avoid exceeding the maximum volume of the vial.
26. Vortex (**do not vortex >10 seconds**) and film seal or cap the titration vials. Label and set aside at RT protected from light.

## Additional Reagent Preparation

27. Use the Reagents Calculation Tables to determine the final volume of 1X Antibody Diluent/Block needed and add to a 6 mL Bond Titration vial.
28. Fill a 30 mL Bond Container with 1X PBS, pH 7.4 and place in the first position of the Bond Research Detection System 2.

## BOND RX PROTOCOL SETUP & STAINING

### Add Reagents to BOND RX Program List

29. Some reagents do not need to be added to the software program and can be added directly to the protocol. For reagents that need to be added to the software program, select “Ancillary” in the drop-down box and select the “Preferred” box to ensure the reagents will be included on the default list. Select “Hazardous” when adding the 4% PFA and the H<sub>2</sub>O<sub>2</sub> +Alcohol Solution to ensure waste is routed to the correct container.

Reagents to be added to the BOND RX software include the following:

- Barcode 1
- Barcode 2
- Barcode 3
- Barcode 4
- Barcode 5
- Barcode 6
- H<sub>2</sub>O<sub>2</sub> + Alcohol
- Antibody Cocktail
- 4% PFA
- Buffer

**NOTE** Use 1X PBS, pH 7.4 as Buffer in the 30 mL container paired with the Leica BOND Research Detection System 2.

### Create BOND RX Protocol

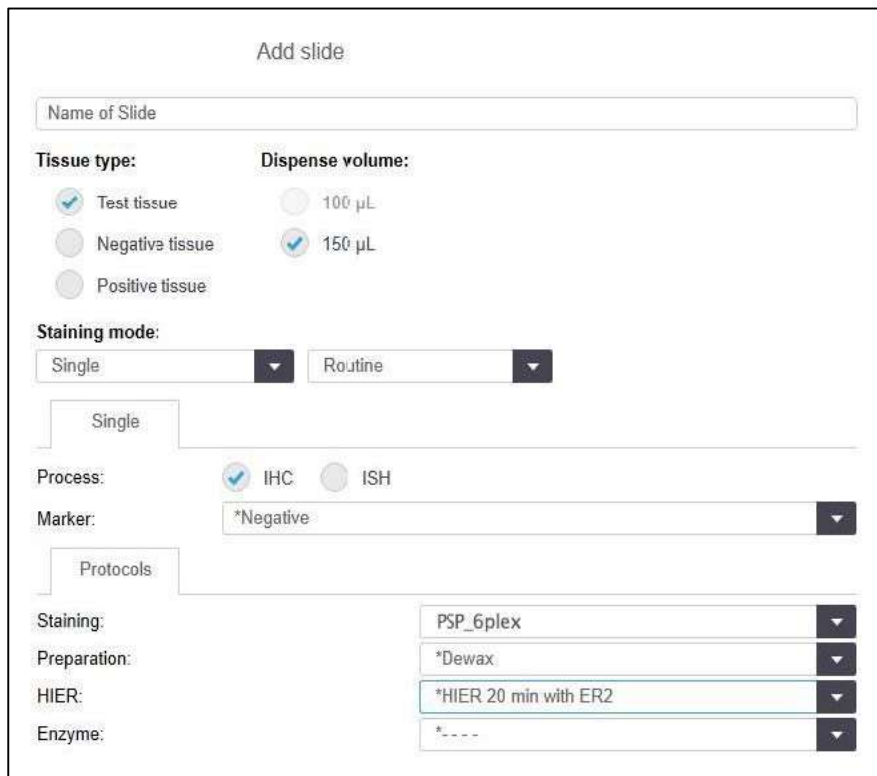
30. Create the PSP\_6plex protocol in the BOND RX software using the included step-by-step instructions. Copy the preprogrammed IF protocol and delete all steps to begin building the PSP\_6plex protocol. Carefully select each wash and reagent step as indicated; some steps are set up as “open” or “intermediate” dispense type, and some use heat. Add reagent or wash steps in accordance with the template. There are 171 steps in the PSP\_6plex protocol.

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## Create Slide Labels to Run BOND RX Protocol

31. Add slides to the study in Slide Setup using the for Preparation and HEIR shown in Figure 2 below.
32. When finished adding slides, print labels for the current study.
  - Name of Slide: Example: ID#-IP-CD4-6plex
  - Tissue Type: Test tissue
  - Dispense Volume: 150 µL
  - Staining Mode: Single, Routine
  - Process: IHC
  - Marker: \*Negative
  - Protocols:
    - o Staining: PSP\_6plex
    - o Preparation: \*Dewax
    - o HIER: \*HIER 20 min with ER2
    - o Enzyme: \*---- (no enzyme protocol used)



The screenshot shows a web form titled "Add slide" with the following fields and options:

- Name of Slide:** A text input field.
- Tissue type:** Radio buttons for "Test tissue" (checked), "Negative tissue", and "Positive tissue".
- Dispense volume:** Radio buttons for "100 µL" and "150 µL" (checked).
- Staining mode:** Two dropdown menus, the first set to "Single" and the second to "Routine".
- Process:** Radio buttons for "IHC" (checked) and "ISH".
- Marker:** A dropdown menu set to "\*Negative".
- Protocols:** A section with five dropdown menus:
  - Staining:** PSP\_6plex
  - Preparation:** \*Dewax
  - HIER:** \*HIER 20 min with ER2
  - Enzyme:** \*----

**FIGURE 2. Add Slide Information and Pretreatment Protocol.**

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### Preparing & Running the BOND RX

33. Place labeled slides in slide trays, place Covertiles on top of each slide, and load onto BOND RX.
34. Collect all prepared reagents and place them in the affiliated Bond Containers. Scan to check the prepared reagents have been entered correctly into the software and the container has sufficient volume for the run.
35. Insert all Bond Containers into reagent trays and load on the BOND RX.
36. Ensure all required bulk reagents are at least half full:
  - 1X Bond Wash Solution (prepared from 10X Concentrate)
  - Dewax Solution
  - Bond Epitope Retrieval Solution 1
  - Bond Epitope Retrieval Solution 2
  - ddH<sub>2</sub>O
  - Reagent Grade Alcohol
37. Once all reagents and samples are loaded onto the BOND RX, choose “Delayed Start” to set a start time for the protocol. The 6-plex protocol takes approximately 12.5 hours to complete. Scheduling an overnight run is recommended.
38. When the run is complete, take slide trays off the BOND RX, carefully remove Covertiles, and tap slides to remove excess water.
39. Apply fluorescent-compatible, hard-set, DAPI-free mounting medium and coverslip with a #1.5 glass coverslip. Allow mounting medium to dry for a minimum of 20 minutes prior to imaging with the Phenolmager HT.
40. Perform cleaning for the BOND RX components.
  - Soak and rinse Covertiles with ddH<sub>2</sub>O followed by soaking for 15 min in 100% reagent grade alcohol.
  - Wipe down heating pads.
  - Wipe down slide and reagent trays.

### Reagents Calculation Table(5 Slides)

Calculated component volumes based on Table 1: Panel Setup and Recommended Antibody Dilutions

<b>NUMBER OF SLIDES:</b>					<b>5</b>
<b>BOND</b>	<b>Reagent Name in BOND</b>	<b>Reagent</b>	<b>Dilution</b>	<b>Component</b>	<b>Reagent</b>
<b>Vial</b>	<b>Software</b>		<b>Factor</b>	<b>Volume (µL)</b>	<b>Volume (µL)</b>
6 mL	Akoya Blocking Buffer	1X Antibody Diluent/Block	N/A	<b>1050</b>	1050
<b>Antibody Cocktail Preparation</b>					
N/A	Antibody Cocktail Diluent (not added to BOND software)	N Blocker J Blocker S Blocker PhenoCode Signature Blocker 1 1X Antibody Diluent/Block	1:42 1:42 1:42 1:42 N/A	<b>35.7</b> <b>35.7</b> <b>35.7</b> <b>35.7</b> <b>1357.1</b>	1500
6 mL	Antibody Cocktail	PanCK - BX066 (1:100 diluted stock, 1 µL into 99 µL) CD3e - BX080 (1:100 diluted stock, 1 µL into 99 µL) CD68 - BX015 (1:100 diluted stock, 1 µL into 99 µL) <b>VARIABLE</b> CD20 - BX064 (concentrate) CD8 - BX026 (1:100 diluted stock, 1 µL into 99 µL) Antibody Cocktail Diluent	1:4000 1:12000 1:8000 <b>1:100</b> 1:800 1:2000 N/A	<b>26.3</b> <b>8.8</b> <b>13.1</b> <b>10.5</b> <b>1.3</b> <b>52.5</b> <b>937.6</b>	1050
<b>Barcode Preparation</b>					
N/A	Detector Diluent (not added to BOND software)	PhenoCode Signature Blocker 5 PhenoCode Signature Diluent	1:10	<b>650.0</b> <b>5850.0</b>	6500
6 mL	Barcode 1	HRP-HX066 Detector Diluent	1:100	<b>10.5</b> <b>1039.5</b>	1050
6 mL	Barcode 2	HRP-HX080 Detector Diluent	1:100	<b>10.5</b> <b>1039.5</b>	1050
6 mL	Barcode 3	HRP-HX015 Detector Diluent	1:100	<b>10.5</b> <b>1039.5</b>	1050
6 mL	<b>Barcode 4</b>	<b>VARIABLE</b> Detector Diluent	1:100	<b>10.5</b> <b>1039.5</b>	1050
6 mL	Barcode 5	HRP-HX064 Detector Diluent	1:100	<b>10.5</b> <b>1039.5</b>	1050
6 mL	Barcode 6	HRP-HX026 Detector Diluent	1:100	<b>10.5</b> <b>1039.5</b>	1050
<b>Opal Preparation</b>					
6 mL	Opal 480	Opal 480 1X Plus Auto Amp Diluent	1:150	<b>12</b> <b>1788</b>	1800
6 mL	Opal 520	Opal 520 1X Plus Auto Amp Diluent	1:150	<b>12</b> <b>1788</b>	1800
6 mL	Opal 570	Opal 570 1X Plus Auto Amp Diluent	1:100	<b>18</b> <b>1782</b>	1800
6 mL	Opal 620	Opal 620 1X Plus Auto Amp Diluent	1:150	<b>12</b> <b>1788</b>	1800
6 mL	Opal 690	Opal 690 1X Plus Auto Amp Diluent	1:150	<b>12</b> <b>1788</b>	1800
6 mL	Opal TSA-DIG	Opal TSA-DIG 1X Plus Auto Amp Diluent	1:100	<b>18</b> <b>1782</b>	1800
6 mL	Opal 780	Opal 780 1X Antibody Diluent/Block	1:25	<b>42</b> <b>1008</b>	1050
6 mL	Spectral DAPI	10X Spectral DAPI (# drops) 1X PBS	3 drops/mL	<b>5</b> <b>1800</b>	1800

### Reagents Calculation Table(10 Slides)

Calculated component volumes based on Table 1: Panel Setup and Recommended Antibody Dilutions

					NUMBER OF SLIDES:	10
BOND	Reagent Name in BOND	Reagent	Dilution	Component	Reagent	
Vial	Software		Factor	Volume (µL)	Volume (µL)	
6 mL	Akoya Blocking Buffer	1X Antibody Diluent/Block	N/A	<b>1800</b>	1800	
<b>Antibody Cocktail Preparation</b>						
N/A	Antibody Cocktail Diluent (not added to BOND software)	N Blocker J Blocker S Blocker PhenoCode Signature Blocker 1 1X Antibody Diluent/Block	1:42 1:42 1:42 1:42 N/A	<b>53.6</b> <b>53.6</b> <b>53.6</b> <b>53.6</b> <b>2035.7</b>	2250	
6 mL	Antibody Cocktail	PanCK - BX066 (1:100 diluted stock, 1 µL into 99 µL) CD3e - BX080 (1:100 diluted stock, 1 µL into 99 µL) CD68 - BX015 (1:100 diluted stock, 1 µL into 99 µL) <b>VARIABLE</b> CD20 - BX064 (concentrate) CD8 - BX026 (1:100 diluted stock, 1 µL into 99 µL) Antibody Cocktail Diluent	1:4000 1:12000 1:8000 <b>1:100</b> 1:800 1:2000 N/A	<b>45.0</b> <b>15.0</b> <b>22.5</b> <b>18.0</b> <b>2.3</b> <b>90.0</b> <b>1607.3</b>	1800	
<b>Barcode Preparation</b>						
N/A	Detector Diluent (not added to BOND software)	PhenoCode Signature Blocker 5 PhenoCode Signature Diluent	1:10	<b>1100.0</b> <b>9900.0</b>	11000	
6 mL	Barcode 1	HRP-HX066 Detector Diluent	1:100	<b>18.0</b> <b>1782.0</b>	1800	
6 mL	Barcode 2	HRP-HX080 Detector Diluent	1:100	<b>18.0</b> <b>1782.0</b>	1800	
6 mL	Barcode 3	HRP-HX015 Detector Diluent	1:100	<b>18.0</b> <b>1782.0</b>	1800	
6 mL	<b>Barcode 4</b>	<b>VARIABLE</b> Detector Diluent	1:100	<b>18.0</b> <b>1782.0</b>	1800	
6 mL	Barcode 5	HRP-HX064 Detector Diluent	1:100	<b>18.0</b> <b>1782.0</b>	1800	
6 mL	Barcode 6	HRP-HX026 Detector Diluent	1:100	<b>18.0</b> <b>1782.0</b>	1800	
<b>Opal Preparation</b>						
6 mL	Opal 480	Opal 480 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 520	Opal 520 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 570	Opal 570 1X Plus Auto Amp Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Opal 620	Opal 620 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 690	Opal 690 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal TSA-DIG	Opal TSA-DIG 1X Plus Auto Amp Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Opal 780	Opal 780 1X Antibody Diluent/Block	1:25	<b>72</b> <b>1728</b>	1800	
6 mL	Spectral DAPI	10X Spectral DAPI (# drops) 1X PBS	3 drops/mL	<b>10</b> <b>3300</b>	3300	



### Reagents Calculation Table(20 Slides)

Calculated component volumes based on Table 1: Panel Setup and Recommended Antibody Dilutions

					NUMBER OF SLIDES:	20
BOND Vial	Reagent Name in BOND Software	Component Tube Label	Dilution Factor	Component Volume (µL)	Reagent Volume (µL)	
6 mL	Akoya Blocking Buffer	1X Antibody Diluent/Block	N/A	<b>3300</b>	3300	
<b>Antibody Cocktail Preparation</b>						
N/A	Antibody Cocktail Diluent (not added to BOND software)	N Blocker J Blocker S Blocker PhenoCode Signature Blocker 1X Antibody Diluent/Block	1:42 1:42 1:42 1:42 N/A	<b>89.3</b> <b>89.3</b> <b>89.3</b> <b>89.3</b> <b>3392.9</b>	3750	
6 mL	Antibody Cocktail	PanCK - BX066 (1:100 diluted stock, 1 µL into 99 µL) CD3e - BX080 (1:100 diluted stock, 1 µL into 99 µL) CD68 - BX015 (1:100 diluted stock, 1 µL into 99 µL) <b>VARIABLE</b> CD20 - BX064 (concentrate) CD8 - BX026 (concentrate) Antibody Cocktail Diluent	1:4000 1:12000 1:8000 <b>1:100</b> 1:800 1:2000 N/A	<b>82.5</b> <b>27.5</b> <b>41.3</b> <b>33.0</b> <b>4.1</b> <b>1.7</b> <b>3110.0</b>	3300	
<b>Barcode Preparation</b>						
N/A	Detector Diluent (not added to BOND software)	PhenoCode Signature Blocker 5 PhenoCode Signature Diluent	1:10	<b>2000</b> <b>18000</b>	20000	
6 mL	Barcode 1	HRP-HX066 Detector Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Barcode 2	HRP-HX080 Detector Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Barcode 3	HRP-HX015 Detector Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	<b>Barcode 4</b>	<b>VARIABLE</b> Detector Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Barcode 5	HRP-HX064 Detector Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Barcode 6	HRP-HX026 Detector Diluent	1:100	<b>33</b> <b>3267</b>	3300	
<b>Opal Preparation</b>						
6 mL	Opal 480	Opal 480 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 480	Opal 480 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 520	Opal 520 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 520	Opal 520 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 570	Opal 570 1X Plus Auto Amp Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Opal 570	Opal 570 1X Plus Auto Amp Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Opal 620	Opal 620 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 620	Opal 620 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 690	Opal 690 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal 690	Opal 690 1X Plus Auto Amp Diluent	1:150	<b>22</b> <b>3278</b>	3300	
6 mL	Opal TSA-DIG	Opal TSA-DIG 1X Plus Auto Amp Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Opal TSA-DIG	Opal TSA-DIG 1X Plus Auto Amp Diluent	1:100	<b>33</b> <b>3267</b>	3300	
6 mL	Opal 780	Opal 780 1X Antibody Diluent/Block	1:25	<b>132</b> <b>3168</b>	3300	
6 mL	Spectral DAPI	10X Spectral DAPI (# drops) 1X PBS	3 drops/mL	<b>10</b> <b>3300</b>	3300	
6 mL	Spectral DAPI	10X Spectral DAPI (# drops) 1X PBS	3 drops/mL	<b>10</b> <b>3300</b>	3300	

# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
1 *Bond ER Solution 2	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
2 *Bond ER Solution 2	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
3 *Bond ER Solution 2	Step type: Reagent	Inc. (min): 20:00	Temperature: 100	Dispense type: Intermediate
Step Reagent	Supplier: Leica Microsystems			
4 *Bond ER Solution 2	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
5 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
6 *Bond Wash Solution	Step type: Wash	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
7 *Bond Wash Solution	Step type: Wash	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
8 *Bond Wash Solution	Step type: Wash	Inc. (min): 2:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
9 Buffer	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
10 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
11 H <sub>2</sub> O <sub>2</sub> +Alcohol	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
12 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
13 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL

\* BOND RX — For research use only. Not for use in clinical procedures.

# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
14 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
15 *Deionized Water	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
16 *Bond Wash Solution	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
17 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
18 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya			
19 *Akoya Blocking Buffer	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
20 Antibody Cocktail	Step type: Reagent	Inc. (min): 30:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
21 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
22 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
23 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
24 4% PFA	Step type: Reagent	Inc. (min): 30:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
25 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
26 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
27 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
28 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
29 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
30 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 10:00	Temperature: 60	Dispense type: Intermediate
Step Reagent	Supplier: Leica Microsystems			
31 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
32 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
33 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
34 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
35 *Deionized Water	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
36 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
37 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
38 Barcode 1	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
39 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
40 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
41 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
42 *Bond Wash Solution	Step type: Wash	Inc. (min): 3:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
43 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
44 *Opal 690 Reagent	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
45 *Opal 690 Reagent	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
46 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
47 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
48 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
49 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
50 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
51 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 10:00	Temperature: 60	Dispense type: Intermediate
Step Reagent	Supplier: Leica Microsystems			
52 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
53 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
54 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
55 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
56 *Deionized Water	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
57 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
58 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
59 Barcode 2	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
60 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
61 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
62 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
63 *Bond Wash Solution	Step type: Wash	Inc. (min): 3:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
64 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
65 *Opal 570 Reagent	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
66 *Opal 570 Reagent	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
67 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
68 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
69 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
70 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
71 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
72 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 10:00	Temperature: 60	Dispense type: Intermediate
Step Reagent	Supplier: Leica Microsystems			
73 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
74 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
75 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
76 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
77 *Deionized Water	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
78 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
79 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
80 Barcode 3	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
81 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
82 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
83 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
84 *Bond Wash Solution	Step type: Wash	Inc. (min): 3:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
85 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya			
86 *Opal TSA-DIG	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya			
87 *Opal TSA-DIG	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
88 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
89 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
90 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
91 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
92 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
93 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 10:00	Temperature: 60	Dispense type: Intermediate
Step Reagent	Supplier: Leica Microsystems			
94 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
95 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
96 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
97 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
98 *Deionized Water	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
99 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
100 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
101 Barcode 4	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
102 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
103 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
104 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

\* BOND RX — For research use only. Not for use in clinical procedures.

# PROTOCOL

## PSP\_6plex

Full name	PhenoCode Signature Panel 6-plex
ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
105 *Bond Wash Solution	Step type: Wash	Inc. (min): 3:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
106 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
107 *Opal 520 Reagent	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
108 *Opal 520 Reagent	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
109 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
110 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
111 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
112 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
113 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
114 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 10:00	Temperature: 60	Dispense type: Intermediate
Step Reagent	Supplier: Leica Microsystems			
115 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
116 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
117 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

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ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
118 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
119 *Deionized Water	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
120 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
121 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
122 Barcode 5	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
123 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
124 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
125 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
126 *Bond Wash Solution	Step type: Wash	Inc. (min): 3:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
127 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
128 *Opal 620 Reagent	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
129 *Opal 620 Reagent	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
130 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

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ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
131 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
132 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
133 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
134 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
135 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 10:00	Temperature: 60	Dispense type: Intermediate
Step Reagent	Supplier: Leica Microsystems			
136 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
137 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
138 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
139 *Bond Wash Solution	Step type: Wash	Inc. (min): 8:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
140 *Deionized Water	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
141 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
142 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
143 Barcode 6	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

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ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
144 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
145 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
146 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
147 *Bond Wash Solution	Step type: Wash	Inc. (min): 3:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
148 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya			
149 *Opal 480 Reagent	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya			
150 *Opal 480 Reagent	Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
151 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
152 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
153 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
154 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
155 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
156 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 10:00	Temperature: 60	Dispense type: Intermediate

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# PROTOCOL

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Type	IHC staining
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Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

BOND RX				
Step Reagent	Supplier: Leica Microsystems			
157 *Bond ER Solution 1	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
158 *Bond Wash Solution	Step type: Wash	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
159 *Bond Wash Solution	Step type: Wash	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Not applicable			
160 *Deionized Water	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
161 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
162 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya			
163 *Opal 780 Reagent	Step type: Reagent	Inc. (min): 60:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
164 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
165 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
166 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
167 *Spectral DAPI	Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Akoya (previously Perkin Elmer)			
168 *Spectral DAPI	Step type: Reagent	Inc. (min): 5:00	Temperature: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
169 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

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# PROTOCOL

## PSP\_6plex

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ID	X.XXX
Type	IHC staining
Created by	bondpoweruser
Creation time	MM/DD/YYYY HH:MM:SS AM/PM
Facility	Akoya R&D
Staining method	Single

### BOND RX

Step Reagent	Supplier: Leica Microsystems			
170 *Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
171 *Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

#### TRADEMARKS

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