

Staining Kit for PhenoCode Signature

CATALOG # PCSP0100

OVERVIEW

Create custom multiplexed PhenoCode™ Signature assays for human formalin-fixed, paraffin-embedded (FFPE) tissue samples using the Staining Kit for PhenoCode Signature. Simultaneously multiplex up to 6 antibodies of interest to create a novel panel using this kit with PhenoCode Signature antibodies and Opal® dyes (both sold separately) for high sensitivity biomarker detection in up to 20 human FFPE tissue slides (maximum 4 batches of 5 slides). Stain slides using the Leica® BOND RX™ autostainer with software v6 or above, and image slides using an Akoya Phenolmager® system. If using a custom conjugated antibody (see Antibody Conjugation Kit for PhenoCode Signature, Catalog # PCSP0200), conjugation should be performed at least 2 days prior to use in PhenoCode Signature assays to avoid high levels of nuclear background staining. Standard hydration and antigen retrieval processes are included in this protocol.

Experiment Overview

After dewaxing, epitope retrieval, and blocking, tissue sections are stained with the PhenoCode Signature antibody cocktail. Antibodies are conjugated to unique oligo barcodes and applied to tissue in a single cocktail incubation step. 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.

MATERIALS PROVIDED

Component #	Component Name	Units	Shipment & Storage Temps
200035	1X Antibody Diluent/Block	1	4°C
FP1609	1X Plus Automation Amplification Diluent	1	
PCSA1001	PhenoCode Signature Diluent	1	
232108	N Blocker	1	
232110	J Blocker	1	
232111	S Blocker	1	-20°C
200039	PhenoCode Signature Blocker 1	1	
200040	PhenoCode Signature Blocker 5	2	

NOTE A Calculations Worksheet is appended to the end of this document as an aid in calculating reagent volumes where indicated by prompts  for corresponding steps throughout the protocol.

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MATERIALS NOT PROVIDED

Required to prepare 20 slides:

	Item	Recommended Vendor Minimum Volume	Recommended Vendor Part Number
Laboratory Materials	PhenoCode Signature Antibodies	Akoya Biosciences	S650XXX
	Custom-Conjugated Antibody (optional)		PCSP0200
	Opal Reagent Packs		Opal 480: FP1500001KT Opal 520: FP1487001KT Opal 570: FP1488001KT Opal 620: FP1495001KT Opal 690: FP1497001KT Opal 780: FP1501001KT
	10X Spectral DAPI		FP1490
	30% Hydrogen Peroxide (H ₂ O ₂)	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 ₂ O)	N/A	N/A
	Mounting media: fluorescent-compatible, hard-set, DAPI-free	Thermo Fisher ProLong™ Diamond Antifade Mountant	P36965
	Glass coverslips, #1.5	N/A	N/A
	Vortex	N/A	N/A
	Tabletop Centrifuge	N/A	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 Titration Kit		OPT9049
	Bond Slide Label and Print Ribbon Kit		Several Options
	Bond Universal Covertiles		S21.4611
	Wash Solution 10X Concentrate		AR9590
	Dewax Solution		AR9222

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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). Prepared reagents can be stored at RT in Bond Titration vials until being loaded onto the BOND RX. Remove film seals or caps on the titration vials prior to loading onto the autostainer later in the day. Refer to PhenoCode Signature antibody Technical Datasheets for recommended antibody starting point dilutions. Individual tissue properties and the inherent variance across tissue types can influence panel optimization and the amount of each antibody required for detection.

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 1. Volumes in the table are based on total number of slides run. BOND RX dead volumes are accounted for in Table 1.
3. Label and film seal or cap the titration vial. Set aside at RT.

TABLE 1. 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. Use Table 2 to determine the total volume of Alcohol Solution needed; consider total number of slides to be run including experimental, control, and autofluorescence slides.

4. Prepare 3% H₂O₂ in 50% alcohol using a 6 mL Bond Titration vial under a fume hood using the guidelines in Table 2 (BOND RX dead volumes are accounted for in the table).
5. Label and film seal or cap the titration vial. Set aside at RT.

TABLE 2. Guidelines for Preparing Alcohol Solution

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

Additional Reagent Preparation

6. Calculate the volume of 1X Antibody Diluent/Block needed and add to a 6 mL Bond Titration vial.
7. Fill a 30 mL Bond Container with 1X PBS, pH 7.4. Place in the first position of the Bond Research Detection System 2.



Antibody Cocktail Preparation

8. Allow S Blocker and PhenoCode Signature Blocker 1 to thaw and equilibrate to RT.
9. Retrieve N Blocker, J Blocker, and 1X Antibody Diluent/Block from 4°C storage.
10. Vortex the following blocking reagents (**do not vortex >3 seconds**) and spin down for 15-30 seconds:
 - N Blocker
 - J Blocker
 - S Blocker
 - PhenoCode Signature Blocker 1

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11. Prepare the Antibody Cocktail Diluent by adding each of the above blocking reagents to 1X Antibody Diluent/Block.
 - a. Calculate the total volume of the Antibody Cocktail Diluent.
 - b. Calculate the volume per blocking reagent (N, J, S, PhenoCode Signature Blocker 1).
 - c. Calculate the total volume of all 4 blocking reagents.
 - d. Calculate the volume of 1X Antibody Diluent/Block to use in the Antibody Cocktail Diluent.
 - e. Vortex briefly (**do not vortex >3 seconds**) or gently mix by inverting several times.

12. Prepare antibodies to use in the Antibody Cocktail; Table 3 can be used as an aid. Gently mix each tube of concentrated antibody stock by inverting several times or vortex (**do not vortex >3 seconds**), then spin down for 15-30 seconds.
 - a. Calculate the volume of Antibody Cocktail.
 - b. Determine the dilution of each antibody. Recommended starting dilutions can be found in Technical Data Sheets for PhenoCode Signature Antibodies. See NOTE below on performing dilutions.
 - c. Calculate the volume of each antibody solution (diluted antibody made from concentrated antibody stock or direct use of concentrated antibody stock) to add to the Antibody Cocktail.
 - d. Calculate the total volume of antibody solutions.
 - e. Calculate the volume of Antibody Cocktail Diluent and add to a 6 mL Bond Titration vial, then add each antibody solution to create the Antibody Cocktail.
 - f. 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.

NOTE: If the calculated volume of concentrated antibody stock is less than 1 μL , use a 2-step dilution process:

Step 1 Prepare a 1:100 dilution: use 1 μL concentrated antibody stock in 99 μL Antibody Cocktail Diluent.

Step 2 Create the final dilution: use a portion of the freshly prepared 1:100 solution to further dilute the antibody in the Antibody Cocktail.

Example: To prepare 5 slides for staining, 1050 μL final volume of Antibody Cocktail is needed. The recommended starting dilution for the antibody is 1:12000.

Step 1 Use 1 μL of concentrated antibody stock to dilute in 99 μL Antibody Cocktail Diluent (1:100 dilution).

Step 2 Dilute the 1:100 solution to 1:12000 by using a 1:120 dilution. Use 8.8 μL of the 1:100 diluted antibody solution in the Antibody Cocktail Diluent (1050 μL total Antibody Cocktail volume needed for 5 slides).

TABLE 3. Antibody Dilutions for use in Antibody Cocktail

	Volume	Antibody Dilution	1-Step Dilution	2-Step Dilution	
	(# of Slides x 150 μL) + 300 μL		Antibody Volume	1 st Dilution (1:100)	2 nd Dilution
Antibody Cocktail					
Antibody 1					
Antibody 2					
Antibody 3					
Antibody 4					
Antibody 5					
Antibody 6					

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Detector Preparation

13.

Allow PhenoCode Signature Blocker 5, PhenoCode Signature Diluent, and PhenoCode Signature Detectors (ex. HRP-HX026, HRP-HX067, etc.) to thaw and equilibrate to RT.
- NOTE**

Do not exceed 5 freeze-thaw cycles of PhenoCode Signature Detectors.
14.

Gently mix each PhenoCode Signature Detector by pipetting up and down **(do not vortex)**, then spin down for 15-30 seconds.
15.

Calculate the volume of Detector Diluent needed.
16.

Prepare Detector Diluent by diluting PhenoCode Signature Blocker 5 at 1:10 in PhenoCode Signature Diluent.

a.

Calculate the volume of PhenoCode Signature Blocker 5.

b.

Calculate the volume of PhenoCode Signature Diluent.
17.

Use 6 mL Bond Titration vials to prepare each Detector Working Solution at a 1:100 dilution by combining each PhenoCode Signature Detector with the Detector Diluent prepared above.

a.

Calculate the volume of Detector Working Solution per PhenoCode Signature Detector.

b.

Calculate the volume of each PhenoCode Signature Detector.

c.

Calculate the volume of the Detector Diluent per PhenoCode Signature Detector.
18.

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.
19.

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, then skip to Step 22.
20.

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-DIC	Opal 780
Opal Dye Reconstitution							
Diluent Volume (μL)	75	75	75	75	75	75	300
Diluent	DMSO						ddH ₂ O
Opal Dye Working Solution							
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

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21. Vortex all reconstituted Opal dyes (**do not vortex >10 seconds**) and spin down to create Opal dye stock solutions. Wait 15 minutes for the Opal to dissolve, then repeat the vortex and spin down process one time.
22. To create working solutions of each Opal dye (except Opal 780), dilute the Opal dye stock solutions in 1X Plus Automation Amplification Diluent using the dilution factors in Table 4. Calculate the total volume of working solution of each Opal dye. 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. 
23. To create Opal 780 working solution, dilute the Opal 780 stock solution in 1X Antibody Diluent/Block. 
24. Vortex (**do not vortex >10 seconds**) and film seal or cap the titration vials. Label and set aside at RT protected from light.

Spectral DAPI Preparation

25. Add 3 drops of 10X Spectral DAPI solution per 1 mL of 1X PBS. 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.

BOND RX PROTOCOL SETUP & STAINING

Add Reagents to BOND RX Program List

27. 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 H₂O₂+ 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
- H₂O₂ + Alcohol
- Antibody Cocktail
- 4% PFA
- Buffer

NOTE Use 1X PBS, pH 7.4 as Buffer in the 30 mL Bond Container paired with the Leica BOND Research Detection System 2. Number of Barcodes is based on desired plex size.

Create BOND RX Protocol

28. Create the PSP_6plex or PSP_5plex 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 or PSP_5plex 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. There are 171 steps in the PSP_6plex protocol and 150 steps in the PSP_5plex protocol.

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Create Slide Labels to Run BOND RX Protocol

29. Add slides to the study in Slide Setup using the settings for Preparation and HEIR shown in Figure 1.

30. When finished adding slides, print labels for the current study.

- Name of Slide: Example: ID#-PSP-NOVEL-6plex
- Tissue Type: Test tissue
- Dispense Volume: 150 µL
- Staining Mode: Single, Routine
- Process: IHC
- Marker: *Negative
- Protocols:
 - Staining: PSP_6plex or PSP_5plex
 - Preparation: *Dewax
 - HIER: *HIER 20 min with ER2
 - Enzyme: *---- (no enzyme protocol used)

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

- Name of Slide:** A text input field.
- Tissue type:** Radio buttons for "Test tissue" (selected), "Negative tissue", and "Positive tissue".
- Dispense volume:** Radio buttons for "100 µL" and "150 µL" (selected).
- Staining mode:** Two dropdown menus, the first set to "Single" and the second to "Routine".
- Process:** Radio buttons for "IHC" (selected) and "ISH".
- Marker:** A dropdown menu set to "*Negative".
- Protocols:** A section with five dropdown menus:
 - Staining:** Set to "PSP_6plex".
 - Preparation:** Set to "*Dewax".
 - HIER:** Set to "*HIER 20 min with ER2".
 - Enzyme:** Set to "*----".

FIGURE 1. Add Slide Information and Pretreatment Protocol.

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Preparing & Running the BOND RX

31. Place labeled slides in slide trays, place Covertiles on top of each slide, and load onto BOND RX.
32. 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.
33. Insert all Bond Containers into reagent trays and load on the BOND RX.
34. 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
 - ddH₂O
 - Reagent Grade Alcohol
35. 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.
36. When the run is complete, take slide trays off the BOND RX, carefully remove Covertiles, and tap slides to remove excess water.
37. 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.
38. Perform cleaning for the BOND RX components.
 - Soak and rinse Covertiles with ddH₂O followed by soaking for 15 min in 100% reagent grade alcohol.
 - Wipe down heating pads.
 - Wipe down slide and reagent trays.

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CALCULATION WORKSHEET

Fill in the worksheet to determine reagent volumes. Prompts for corresponding steps are indicated by  in the protocol.

Step	Reagent Volume of:	Calculation	Calculated Volume
6	1X Antibody Diluent/Block	= (# of slides x 150 µL) + 300 µL	
11.a.	Antibody Cocktail Diluent	= (# of slides x 150 µL) + 750 µL	
11.b.	Each Blocking Reagent (N, J, S, PhenoCode Signature Blocker 1)	= Antibody Cocktail Diluent / 42	
11.c.	Combined Blocking Reagents (N, J, S, PhenoCode Signature Blocker 1)	= Each Blocking Reagent x 4	
11.d.	1X Antibody Diluent/Block (for Antibody Cocktail Diluent)	= Antibody Cocktail Diluent - Combined Blocking Reagents	
12. a.	Antibody Cocktail	= (# of slides x 150 µL) + 300 µL	
12.c.	Each Antibody Solution	= Antibody Cocktail / Antibody Dilution Factor	
12.d.	Combined Antibody Solutions	= ∑ (Each Antibody Solution)	
12.e.	Antibody Cocktail Diluent	= Antibody Cocktail - Combined Antibody Solutions	
15.	Detector Diluent	= Plex Size (Number of Slides x 150 µL + 300 µL) + 200 µL	
16.a.	PhenoCode Signature Blocker 5	= Detector Diluent / 10	
16.b.	PhenoCode Signature Diluent	= Detector Diluent - PhenoCode Signature Blocker 5	
17.a.	Each Detector Working Solution	= # of slides x 150 µL + 300 µL	
17.b.	Each PhenoCode Signature Detector	= Each Detector Working Solution / 100	
17.c.	Each Detector Diluent	= Detector Working Solution - Each PhenoCode Signature Detector	
22	Each Opal Dye Working Solution (except Opal 780)	= (# of slides x 300 µL) + 300 µL	
22	Each Opal Dye Working Solution if preparing ≥17 slides (except Opal 780)	= (# of slides x 300 µL) + 600 µL	
22	Each Opal Dye Stock Solution	= Each Opal Dye Working Solution / Dilution Factor	
23	Opal 780 Working Solution	= (# of slides x 150 µL) + 300 µL	
23	Opal 780 Stock Solution	= Opal 780 Working Solution / Dilution Factor	
23	1X Antibody Diluent/Block (for Opal 780)	= Opal 780 Working Solution – Opal 780 Stock Solution	
25	DAPI	= (# of slides x 300 µL) + 300 µL NOTE: round up to nearest 1 mL	
25	DAPI if preparing ≥17 slides	= (# of slides x 300 µL) + 600 µL NOTE: round up to nearest 1 mL	

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 ₂ O ₂ +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

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

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

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

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

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

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

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

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

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			
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

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			
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

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			
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

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			
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

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			
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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