

Opal™ Polaris 7-Color Automation IHC Kit 50 slides (NEL871001KT)

PRODUCT INFORMATION

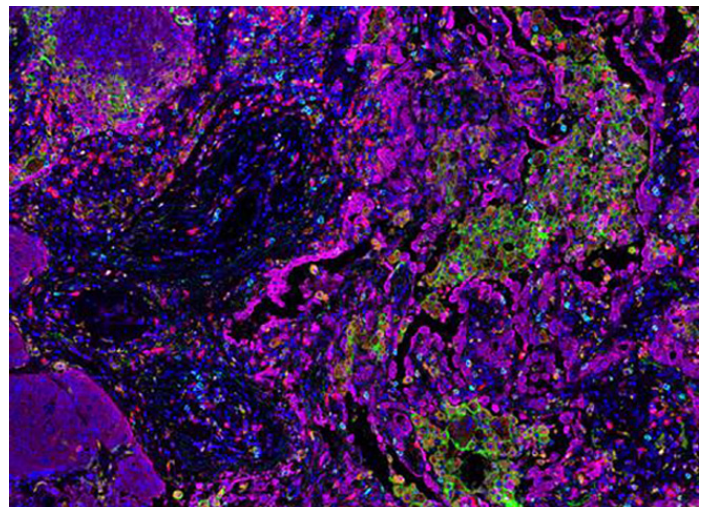
Storage	Store kits in the dark at 4°C.
Stability	See kit label on outside of box for expiration date
Application	The Opal Polaris 7-Color kits are intended for multiplex fluorescent IHC.
User Manual	Download the latest version of the User Manual at www.akoyabio.com/opal
Safety Note	DMSO is classified as hazardous and combustible. Some reagents in this kit contain Proclin® 300 that is classified as corrosive to metals and skin, a skin and eye irritant, and hazardous to the aquatic environment. DAPI is considered corrosive to the skin and an irritant to the eye. All other reagents are classified as nonhazardous. It is strongly recommended to wear disposable gloves and safety glasses while working with the items in this kit. Thorough washing of hands after handling is also recommended.
Quality Control	We certify that QC results of these reagents meet our quality release criteria.

WHAT IS THE OPAL METHOD?

The Opal workflow allows simultaneous detection of multiple biomarkers in tissue. This Opal protocol was written specifically for Opal Polaris 7 -Color Automation IHC on formalin fixed paraffin embedded (FFPE) tissue* using Leica BOND RX. The approach involves detection with Opal reactive fluorophores, followed by heat-induced stripping steps for: removal of primary and secondary antibodies; removal of any non-specific staining; and reduction of tissue auto-fluorescence. The Opal signal is largely unaffected by stripping and antibody removal. After stripping, another round of staining can be performed for additional target detection without risk of antibody cross reactivity. Please pay additional attention to Opal Polaris 780: since it's an antibody based staining step, stripping can **NOT** be performed after Opal Polaris 780 binds to the tissue. Thus, please assign Opal Polaris 780 staining to the last cycle of your staining protocol..

Opal allows staining of multiple IHC targets using unlabeled primary antibodies raised in the same species². Combining Opal with multispectral imaging and analysis enables simultaneous, quantitative results for up to 6 biomarkers in fluorescence, even with co-localized markers, plus nuclear counterstain (DAPI). Opal Polaris 7-Color assay is specially designed to work with Vectra Polaris imaging system. **It is not compatible with Vectra® 3 and may need additional components for imaging on Mantra.**

*Please contact us if you would like to work with other types of samples. Akoya Biosciences provides assistance with assay development and offers multiplex Opal IHC and IF services. Visit: www.akoyabio.com/Opal.



Human Tonsil section was stained with Opal Polaris Color IHC Kit and imaged on the Vectra Polaris

Target	Color
CD8	Opal Polaris 480
PD-L1	Opal 520
FoxP3	Opal 570
CD68	Opal 620
PD-1	Opal 690
PanCK	Opal Polaris 780

MATERIAL PROVIDED			
	Format*	Product #	Kit Components
Opal Polaris 7-Color Automation IHC Kit	50 slides	NEL871001KT	<ul style="list-style-type: none"> • 1 X Plus Automation Amplification Diluent (2 X 50mL) • Opal Polaris 480 Fluorophore (X1) • Opal 520 Fluorophore (X1) • Opal 570 Fluorophore (X1) • Opal 620 Fluorophore (X1) • Opal 690 Fluorophore (X1) • Opal Polaris 780 Fluorophore (X1) <ul style="list-style-type: none"> - a. Opal TSA-DIG (X1) - b. Opal Polaris 780 (X1) • DMSO (1 X 500 µL) • Spectral DAPI solution (1 X 1.5 mL) • Blocking/Ab Diluent (1 X 100 mL) • Opal Polymer HRP Ms + Rb (2 X 50 mL)
*The format of the kit is based on ~150 µL per slide of Opal Working Solution.			

SOLUTIONS TO PREPARE

Primary Antibody Working Solution

Dilute primary antibody in PerkinElmer Antibody Diluent / Block at optimal concentration for Opal detection as determined below.

Secondary Antibody Working Solution

Opal Polymer HRP Ms + Rb is supplied as a ready-to-use solution and does not need to be optimized for use with Opal fluorophores.

Opal Working Solution

Reconstitute each Opal Fluorophore (with the exception of **Opal Polaris 780**) in 75 µL of DMSO. Before each procedure, dilute Opal Fluorophore in 1X Amplification Diluent to make Opal Fluorophore Working Solution. Recommend to start diluting the Opal Fluorophore at 1:150. Optimize your assay according to the Opal Assay Development Guide.

Opal Polaris 780 Working Solution

Reconstitute Opal TSA-DIG in 75 µL of DMSO, and Opal

Polaris 780 in 300 µL of water. Before the procedure, dilute Opal TSA-DIG in 1X Amplification Diluent at 1:100 to make Opal TSA-DIG Working Solution. Dilute Opal Polaris 780 with Ab diluent/blocking at 1:25 to make a working solution.

DAPI working solution

Add three drops of DAPI solutions into 1ml of TBS. Approximately 150 µL of DAPI Working Solution is required per slide. Discard any unused portion of DAPI Working Solution.

SPECIAL CONSIDERATIONS AND BOND RX PROTOCOL

Opal Polaris 780 Automation Steps

The Opal Polaris 780 reaction is antibody based. Because of this, there must be additional washing steps to cool down the slide between the Opal TSA DIG stripping step and the Opal Polaris 780 application step. Please find the Opal Polaris 7 Color BOND RX Protocol [here](#).

IHC TROUBLESHOOTING	
PROBLEM	REMEDY
Low Signal	<ul style="list-style-type: none"> • Titer primary and/or Opal dyes to determine optimum concentration for Opal detection . • Increase primary antibody and/or Opal Working Solution incubation time . • Change staining order of multiplex and move this specific target detection towards the end of staining procedure.
Excess Signal	<ul style="list-style-type: none"> • Decrease concentration of primary antibody . • Decrease concentration of Opal dyes . • Decrease Opal Working Solution incubation time. (Suggested range is 3-10 minutes.)
High Background	<ul style="list-style-type: none"> • Add extra step to block endogenous peroxidases . • Titer primary and/or Opal dyes to determine optimum concentration for Opal detection . • Make fresh buffers . • Evaluate laboratory water source for contamination . • Increase number and/or length of washes .

Opal Fluorophore Excitation and Emission Maxima			
Fluorophore	Excitation	Emission	Cap color
Spectral DAPI	368 nm	461 nm	Blue Dropper
Opal Polaris 480	450 nm	500 nm	Purple
Opal 520	494 nm	525 nm	Green
Opal 570	550 nm	570 nm	Red
Opal 620	588 nm	616 nm	Brown
Opal 690	676 nm	694 nm	Clear
Opal Polaris 780	750 nm	770 nm	Orange

RELATED PRODUCTS

Opal Multiplex IHC Detection Kits	Sizes	Product #
Opal 4-Color Automation IHC Kit*	50 slides	NEL820001 KT
Opal 7-Color Automation IHC Kit*	50 slides	NEL821001KT
Opal 4-Color Manual IHC Kit	50 slides	NEL810001KT
Opal 7-Color Manual IHC Kit	50 slides	NEL811001KT
Opal 4 Lymphocyte Kit	50 slides	OP4L Y2001 KT
Opal 7 Immunology Discovery Kit	50 slides	OP7DS2001 KT
Opal 7 Tumor Infiltrating Lymphocyte Kit	50 slides	OP7TL3001 KT
Opal 7 Solid Tumor Immunology Kit	50 slides	OP7TL4001 KT

*Optimized for Leica Biosystems BOND RX System

Opal Fluorophore Reagent Packs	Product #
Opal Polaris 480	FP1500001KT
Opal 520	FP1487001KT
Opal 540	FP1494001KT
Opal 570	FP1488001KT
Opal 620	FP1495001KT
Opal 650	FP1496001KT
Opal 690	FP1497001KT
Opal Polaris 780	FP1501001KT

Ancillary	Product #
1X Plus Automation Amplification Diluent 1 X 50 mL	FP1609
AR6 buffer (1 OX) 4 x 250 mL	AR6001KT
AR6 buffer (1 OX) 250 m L	AR600250ML
AR9 buffer (1 OX) 4 x 250 mL	AR9001KT
AR9 buffer (1 OX) 250 m L	AR900250ML
Antibody Diluent I Block 100 mL	ARD1001EA
Opal Polymer HRP Ms + Rb 50 mL	ARH1001EA
Spectral DAPI	FP1490

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