

Research Use Only. Not for use in diagnostic procedures.

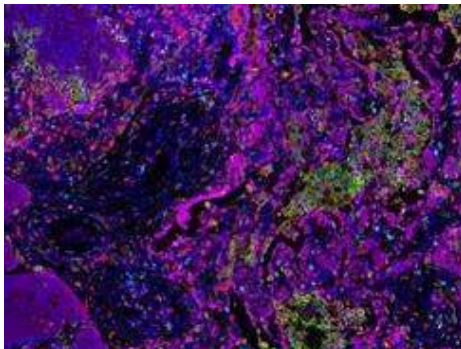
## Opal™ Polaris 7-Color Automation IHC Kit 50 slides (NEL871001KT)

Optimized for Leica Biosystems BOND RX System

### Product Information

<b>Storage</b>	Store kits in the dark at 4°C.
<b>Stability</b>	See kit label on outside of box for expiration date
<b>Application</b>	The Opal Polaris 7-Color kits are intended for multiplex fluorescent IHC.
<b>User Manual</b>	Download the latest version of the User Manual at <a href="http://www.perkinelmer.com/opal">www.perkinelmer.com/opal</a>
<b>Safety Note</b>	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.
<b>Quality Control</b>	We certify that QC results of these reagents meet our quality release criteria.

### What is the Opal Method?



Human Tonsil section was stained with Opal Polaris 7-Color IHC Kit and imaged on the Vectra Polaris

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<sup>1</sup>; 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 species<sup>2</sup>. 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.**

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

**\*Please contact us if you would like to work with other types of samples. PerkinElmer provides assistance with assay development and offers [multiplex Opal IHC and IF services](#). Visit: [www.perkinelmer.com/Opal](http://www.perkinelmer.com/Opal).**

## Material Provided

	Format*	Catalog #	Kit Components
Opal Polaris 7-Color Automation IHC Kit	50 slides	NEL871001KT	<ul style="list-style-type: none"> <li>• 1X Plus Automation Amplification Diluent (2 X 50mL)</li> <li>• Opal Polaris 480 Fluorophore (X1)</li> <li>• Opal 520 Fluorophore (X1)</li> <li>• Opal 570 Fluorophore (X1)</li> <li>• Opal 620 Fluorophore (X1)</li> <li>• Opal 690 Fluorophore (X1)</li> <li>• Opal Polaris 780 Fluorophore (X1)               <ul style="list-style-type: none"> <li>a. Opal TSA-DIG (X1)</li> <li>b. Opal Polaris 780 (X1)</li> </ul> </li> <li>• DMSO (1 X 500 µL)</li> <li>• Spectral DAPI solution (1 X 1.5 mL)</li> <li>• Blocking/Ab Diluent (2 X 100 mL)</li> <li>• Opal Polymer HRP Ms + Rb (2 x 50 mL)</li> </ul>

\*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:

<https://www.akoyabio.com/user-manual/sku/NEL871001KT>

## Troubleshooting

### Technical Support Resources

- Email: [global.techsupport@perkinelmer.com](mailto:global.techsupport@perkinelmer.com)
- Telephone
  - USA toll-free: 800-762-4000
  - EU toll-free: 00800 33 29 0000
  - China toll-free: 800 820 5046
  - Local contact numbers: <http://www.perkinelmer.com/corporate/locations>

## IHC Troubleshooting

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

## Opal Fluorophore Excitation and Emission Maxima

Fluorophore	Excitation	Emission	Cap color
Spectral DAPI	368 nm	461 nm	Blue
Opal Polaris 480	450 nm	500 nm	Violet
Opal520	494 nm	525 nm	Green
Opal570	550 nm	570 nm	Red
Opal620	588 nm	616 nm	Amber
Opal690	676 nm	694 nm	Clear
Opal Polaris 780	750 nm	770 nm	Pink

## Related Products

### Opal Multiplex IHC Detection Kits

	SIZES	PRODUCT NUMBER
Opal 4-Color Automation IHC Kit*	50 slides	NEL820001KT
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	OP4LY2001KT
Opal 7 Immunology Discovery Kit	50 slides	OP7DS2001KT
Opal 7 Tumor Infiltrating Lymphocyte Kit	50 slides	OP7TL3001KT
Opal 7 Solid Tumor Immunology Kit	50 slides	OP7TL4001KT

\*Optimized for Leica Biosystems BOND RX System

### Opal Reagents

	SIZES	PRODUCT NUMBER
Opal Polaris 480	50 slides	FP1500
Opal520 Reagent, powder	50 slides	FP1487A
Opal540 Reagent, powder	50 slides	FP1494A
Opal570 Reagent, powder	50 slides	FP1488A
Opal620 Reagent, powder	50 slides	FP1495A
Opal650 Reagent, powder	50 slides	FP1496A
Opal690 Reagent, powder	50 slides	FP1497A
Opal Polaris 780	50 Slides	FP1501
Spectra DAPI	50 slides	FP1490A

### Ancillary

	PRODUCT NUMBER
1X Plus Automation Amplification Diluent 1 X 50 mL	FP1609
AR6 buffer (10X) 4 x 250 mL	AR6001KT
AR6 buffer (10X) 250 mL	AR600250ML
AR9 buffer (10X) 4 x 250 mL	AR9001KT
AR9 buffer (10X) 250 mL	AR900250ML
Antibody Diluent / Block 100 mL	ARD1001A
Opal Polymer HRP Ms + Rb 50 mL	ARH1001A

For the latest product listings, please go to [www.perkinelmer.com/opal](http://www.perkinelmer.com/opal).

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