

Research Use Only. Not for use in diagnostic procedures.

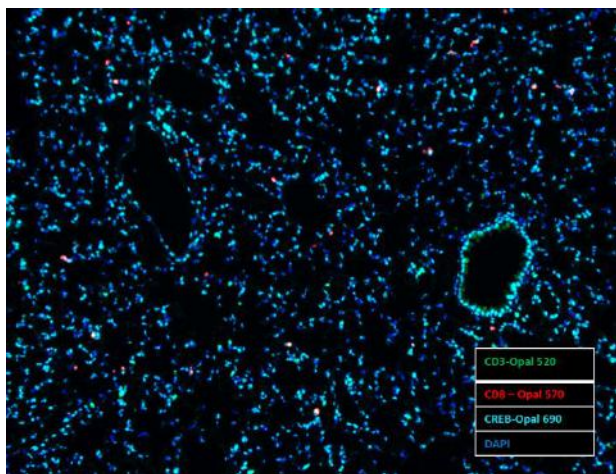
## Opal™ 4-Color anti-Rabbit Automation IHC Kit 50 slides

Optimized for Leica Biosystems BOND RX System

### Product Information

<b>Storage</b>	Store kits in the dark at 4°C.
<b>Stability</b>	This product is stable for a minimum of 3 months when stored at 2–8 °C.
<b>Application</b>	The Opal 4-Color anti-Rabbit Automation IHC kit is 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?



The Opal workflow allows simultaneous detection of multiple biomarkers in tissue. This Opal protocol was written specifically for 4 or 7-Color IHC on formalin fixed paraffin embedded (FFPE) tissue section\*. The approach involves detection with Opal reactive fluorophores, followed by heat-induced stripping 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 step, another round of staining can be performed for additional target detection without risk of antibody cross reactivity.

Opal allows staining of multiple IHC targets using unlabeled primary antibodies raised in the same species. 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). **Fluorescent multispectral imaging (usually with the Mantra™ or Vectra® systems) is required for successful analysis of more than 3 Opal fluorophores at once.**

PerkinElmer provides assistance with assay development and offers [multiplex Opal IHC and IF services](http://www.perkinelmer.com/Opal). Visit: [www.perkinelmer.com/Opal](http://www.perkinelmer.com/Opal).

## Material Provided

	Format*	Catalog #	Kit Components
<b>Opal 4-Color anti-Rabbit Automation IHC Kit</b>	<b>50 slides</b>	<b>NEL830001KT</b>	<ul style="list-style-type: none"> <li>• 1X Plus Automation Amplification Diluent (1 X 50mL)</li> <li>• Opal 520 Fluorophore</li> <li>• Opal 570 Fluorophore</li> <li>• Opal 690 Fluorophore</li> <li>• DMSO (1 X 500 µL)</li> <li>• Spectral DAPI solution (1 X 1.5 mL)</li> <li>• Blocking/Ab Diluent (1 X 100 mL)</li> <li>• Opal Polymer anti-Rabbit HRP (1 x 10 mL)</li> <li>• Opal Polymer anti-Rabbit Diluent (1 x 40 mL)</li> </ul>

\*The format of the kit is based on ~150 µL per slide of Opal Working Solution.

## Usage Instructions

Dilute Opal Polymer anti-Rabbit HRP to the working concentration with provided Opal Polymer anti-Rabbit Diluent.

**Recommended dilution: 1:5**

Other dilutions should be validated.

## 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	Opal Multicolor IHC Kits		Excitation	Emission	Cap color
	4-color	7-color			
Spectral DAPI	✓	✓	358nm	461 nm	Blue
Opal520	✓	✓	494 nm	525nm	Green
Opal540		✓	523nm	536nm	Yellow
Opal570	✓	✓	550 nm	570 nm	Red
Opal620		✓	588 nm	616 nm	Amber
Opal650		✓	627 nm	650 nm	Orange

Opal690	✓	✓	676nm	694 nm	Clear
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## 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-Color anti-Rabbit Automation IHC Kit* 50 Slides		NEL830001KT
Opal 4-Color anti-Rabbit Manual IHC Kit	50 Slides	NEL840001KT
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
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
Spectra DAPI	50 slides	FP1490A

### Ancillary

	PRODUCT NUMBER
1X Plus Automation Amplification Diluent 1 X 50 mL	FP1609
1X Plus Amplification Diluent 1 x 50 mL	FP1498
Opal Polymer anti-Rabbit HRP Kit	ARR1001KT
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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