Automated Multiplex IHC

For Laboratory Use. A research method for research purposes only.

Opal™ 4-Color Automation IHC Kit 50 slides
Opal™ 7-Color Automation IHC Kit 50 slides

Optimized for Leica Biosystems BOND RX System

Product Information

Storage
Store kits in the dark at 4ºC.

Stability
See kit label on outside of box for expiration date

Application
The Opal 4 or 7-Color kits are intended for multiplex fluorescent IHC.

User Manual
Download the latest version of the User Manual at www.perkinelmer.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 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 antibodies¹; 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 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).
Fluorescent multispectral imaging (usually with the Mantra™ or Vectra®
systems) is required for successful analysis of more than 4 Opal
fluorophores at once.

*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.
Material Provided

<table>
<thead>
<tr>
<th>Material Provided</th>
<th>Format*</th>
<th>Catalog #</th>
<th>Kit Components</th>
</tr>
</thead>
</table>
| Opal 4-Color Automation IHC Kit 50 slides | NEL800001KT | • 1X Plus Amplification Diluent (1 X 50 mL)  
• Opal 520 Fluorophore  
• Opal 570 Fluorophore  
• Opal 690 Fluorophore  
• 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 (1 x 50 mL) |
| Opal 7-Color Automation IHC Kit 50 slides | NEL801001KT | • 1X Plus Amplification Diluent (2 X 50 mL)  
• Opal 520 Fluorophore  
• Opal 540 Fluorophore  
• Opal 570 Fluorophore  
• Opal 620 Fluorophore  
• Opal 650 Fluorophore  
• Opal 690 Fluorophore  
• 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.

Troubleshooting

Technical Support Resources
- Email: 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

<table>
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<tr>
<th>PROBLEM</th>
<th>REMEDY</th>
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</table>
| Low Signal  | • 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 | • Decrease concentration of primary antibody.  
• Decrease concentration of Opal dyes.  
• Decrease Opal Working Solution incubation time. (Suggested range is 3-10 minutes.) |
| High Background | • 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

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Opal Multicolor IHC Kits</th>
<th>Excitation</th>
<th>Emission</th>
<th>Cap color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-color</td>
<td>7-color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spectral DAPI</td>
<td>✔</td>
<td>✔</td>
<td>358 nm</td>
<td>461 nm</td>
</tr>
<tr>
<td>Opal520</td>
<td>✔</td>
<td>✔</td>
<td>494 nm</td>
<td>525 nm</td>
</tr>
<tr>
<td>Opal540</td>
<td></td>
<td>✔</td>
<td>523 nm</td>
<td>536 nm</td>
</tr>
<tr>
<td>Opal570</td>
<td>✔</td>
<td>✔</td>
<td>550 nm</td>
<td>570 nm</td>
</tr>
<tr>
<td>Opal620</td>
<td></td>
<td>✔</td>
<td>588 nm</td>
<td>616 nm</td>
</tr>
<tr>
<td>Opal650</td>
<td>✔</td>
<td>✔</td>
<td>627 nm</td>
<td>650 nm</td>
</tr>
<tr>
<td>Opal690</td>
<td>✔</td>
<td>✔</td>
<td>676 nm</td>
<td>694 nm</td>
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# Related Products

## Opal Multiplex IHC Detection Kits

<table>
<thead>
<tr>
<th>Sizes</th>
<th>PRODUCT NUMBER</th>
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<tbody>
<tr>
<td>50 slides</td>
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</table>

- **Opal 4-Color Automation IHC Kit**
  - PRODUCT NUMBER: NEL800001KT
- **Opal 7-Color Automation IHC Kit**
  - PRODUCT NUMBER: NEL801001KT
- **Opal 4-Color IHC Kit**
  - PRODUCT NUMBER: NEL794001KT
- **Opal 5-color fIHC Kit**
  - PRODUCT NUMBER: NEL795001KT
- **Opal 6-color fIHC Kit**
  - PRODUCT NUMBER: NEL796001KT
- **Opal 7-color fIHC Kit**
  - PRODUCT NUMBER: NEL797001KT
- **Opal 4 Lymphocyte Kit**
  - PRODUCT NUMBER: OP4LY1001KT
- **Opal 7 Immunology Discovery Kit**
  - PRODUCT NUMBER: OP7DS1001KT
- **Opal 7 Tumor Infiltrating Lymphocyte Kit**
  - PRODUCT NUMBER: OP7TL1001KT
- **Opal 7 Solid Tumor Immunology Kit**
  - PRODUCT NUMBER: OP7TL2001KT

*Optimized for Leica Biosystems BOND RX System

## Opal Reagents

<table>
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<tr>
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<td>50 slides</td>
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</table>

- **Opal520 Reagent, powder**
  - PRODUCT NUMBER: FP1487A
- **Opal540 Reagent, powder**
  - PRODUCT NUMBER: FP1494A
- **Opal570 Reagent, powder**
  - PRODUCT NUMBER: FP1488A
- **Opal620 Reagent, powder**
  - PRODUCT NUMBER: FP1495A
- **Opal650 Reagent, powder**
  - PRODUCT NUMBER: FP1496A
- **Opal690 Reagent, powder**
  - PRODUCT NUMBER: FP1497A
- **Spectra DAPI**
  - PRODUCT NUMBER: FP1490A

## Ancillary

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- **1X Plus Amplification Diluent 1 X 50 mL**
  - PRODUCT NUMBER: FP1498A
- **AR6 buffer (10X) 4 x 250 mL**
  - PRODUCT NUMBER: AR6001KT
- **AR6 buffer (10X) 250 mL**
  - PRODUCT NUMBER: AR600250ML
- **AR9 buffer (10X) 4 x 250 mL**
  - PRODUCT NUMBER: AR9001KT
- **AR9 buffer (10X) 250 mL**
  - PRODUCT NUMBER: AR900250ML
- **Antibody Diluent / Block 100 mL**
  - PRODUCT NUMBER: ARD1001A
- **Opal Polymer HRP Ms + Rb 50 mL**
  - PRODUCT NUMBER: ARH1001A

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

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