

We've rebranded some of our products. *Phenoptics™* is now *Phenolmager™* and *Vectra® Polaris™* is now *Phenolmager™ HT*.

Opal Polymer Anti-Rabbit HRP Kit, 50mL ARR1001KT

INTENDED USE

Opal Polymer anti-Rabbit HRP Kit is a concentrated immunohistochemistry detection reagent specifically optimized for use with Opal and TSA® Plus detection reagents for maximum sensitivity, reduced incubation time and low background in mouse or human FFPE tissue. This product reacts with primary antibodies raised in rabbit and is part of a rapid, biotin-free detection protocol. Quantitative results may be obtained with optimization of the primary antibody concentration.

MATERIAL PROVIDED

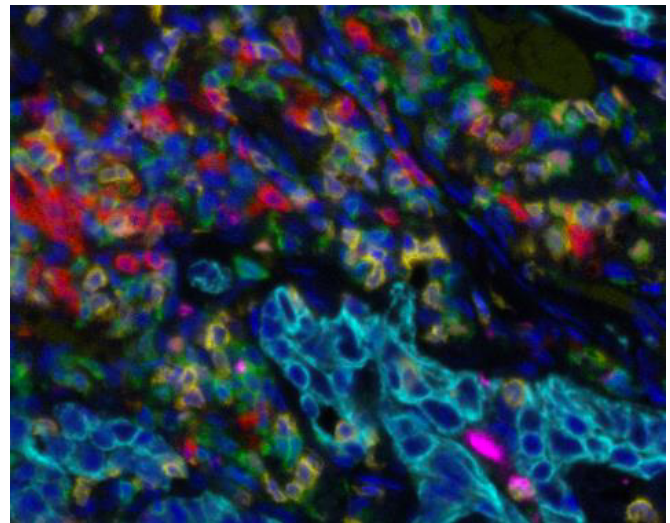
Storage Conditions	Store at 2–8 °C
Description	Opal Polymer anti-Rabbit HRP Kit
Format	50 Slides
Product #	ARR1001KT
Kit Components	·Opal Polymer anti-Rabbit HRP 1x10mL ·Opal Polymer anti-Rabbit HRP Diluent, 1x40 mL

WHAT IS THE OPAL METHOD?

The Opal workflow allows simultaneous detection of multiple biomarkers in tissue. The approach involves detection with Opal fluorophores, followed by microwave treatment (MWT) for: removal of primary and secondary antibodies, removal of any non-specific staining; and reduction of tissue auto-fluorescence. The Opal fluorescence signal is largely unaffected by MWT and antibody removal. After MWT, 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 performed with Phenolmager™ HT (formerly Vectra® Polaris™), Phenolmager Fusion, or Mantra systems) is required for successful analysis of more than 3 Opal fluorophores at once.**

Akoya Biosciences provides assistance with assay development and offers multiplex Opal IHC and IF services. Visit: www.akoyabio.com/phenoimager.



Recommended Detection Protocol

This protocol is written for formal-fixed, paraffin-embedded slides. Please contact us for advice on other types of samples. Deparaffinize slides using standard methods before beginning the detection protocol.

Description	Procedure	Time
1. Slide fixation (optional) with 10% neutral buffered formalin (NBF)	Incubate slide in NBF followed by a distilled water wash to ensure good adhesion of tissue to the slide.	20 min
2. Microwave treatment (MWT) and antigen retrieval.	<ul style="list-style-type: none"> • Rinse slides with AR6 or AR9 working solution as appropriate for the target being detected. • Place slides in an Opal Staining Jar (STJAR4) and fill to the top (~140 mL) with AR6 or AR9 working solution. • Place the slide staining jar into a microwave oven rated at ~1000W near the edge of the carousel and heat on full power until the boiling point is reached (usually about 45 sec). Then continue to heat at 20% power for 15 min. (Risk of fire if the power level is not reduced to 20%. Microwave should not be operated unattended.) • Allow slides to cool to room temperature on the bench for at least 15 minutes. 	32 min
3. Blocking	<ul style="list-style-type: none"> • Rinse slides with water and then with TBST. • Encircle the area of tissue to be stained with a hydrophobic barrier pen. • Apply Antibody Diluent/Block (product number ARD1001EA) to cover the tissue and incubate for 10 minutes at room temperature. (2 drops of Antibody Diluent = ~100 µL) <p>Note: Some mouse tissue samples require alternative blocking methods, such as goat or horse serum. Ensure background is minimal for quantitative results.</p>	10 min
4. Primary antibody incubation	<p>Note: Dilution and incubation time of the primary antibody are points for optimization by the researcher.</p> <ul style="list-style-type: none"> • Apply the primary antibody solution to cover the tissue and incubate. • Rinse slides with TBST and then wash with TBST for 2 min, 3 times. 	30 min to overnight
5. Secondary antibody incubation	<ul style="list-style-type: none"> • Dilute Opal Polymer anti-Rabbit HRP to the working concentration with provided diluent. Recommended dilution: 1:5 • Apply Opal Polymer anti-Rabbit HRP solution to cover the tissue and incubate for 10 minutes at room temperature. (2 drops of Opal Polymer HRP = ~100 µL) • Rinse slides with TBST and then wash with TBST 2 times, 3 minutes each. 	10 min
6. Opal or TSA Plus working solution incubation	<ul style="list-style-type: none"> • Apply Opal fluorophore or TSA Plus working solution to the tissue and incubate for 10 minutes at room temperature. • Wash slides with TBST 3 times, 2 minutes each. 	10 min
7. Microwave treatment (optional)	<p>MWT may be repeated at this point following the procedure outlined in step 2.</p> <p>Reasons for repeating MWT:</p> <ul style="list-style-type: none"> • Remove antibodies to allow multiplexed detection. • Reduce background. <p>MWT has minimal impact on Opal or TSA Plus signal.</p>	32 min
8. Counterstain	<ul style="list-style-type: none"> • Rinse slides in distilled water and then in TBST. • Incubate slides in Spectral DAPI (product number FP1490) solution for five minutes at room temperature. 	5 min
9. Mount	Mount for fluorescence microscopy following the protocol provided by the mounting medium manufacturer.	

Protocol notes

- Do not let slides dry out at any point during the detection protocol.
- A humidified chamber is recommended for all incubation steps.
- Drain off as much of the incubation solutions as possible before the addition of the next solution to prevent reagent dilution and uneven staining. Blot area around, but not on, the tissue section using a tissue.
- Use enough volume of each solution to completely cover the tissue section.

STABILITY

This product is stable for a minimum of 3 months when stored at 2–8 °C.

Opal Multiplex IHC Detection Kits	
Name	Product #
Opal 7-Color Automation IHC Kit*	NEL821001KT
Opal 4-Color Automation IHC Kit*	NEL820001KT
Opal 7-Color Manual IHC Kit	NEL811001KT
Opal 4-Color Manual IHC Kit	NEL810001KT
Detection Reagents	
Opal 4-Color anti-Rabbit Automation IHC Kit*	NEL830001KT
Opal 4-Color anti-Rabbit Manual IHC Kit	NEL840001KT
Opal 4 Lymphocyte Kit	OP4LY2001KT
Opal 7 Immunology Discovery Kit	OP7DS2001KT
Opal 7 Tumor Infiltrating Lymphocyte Kit	OP7TL3001KT
Opal 7 Solid Tumor Immunology Kit	OP7TL4001KT
* for automated workflow with Leica Bond Rx"	

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

Ancillary	
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

HAZARD WARNING

This product is considered to be non-hazardous. Although the product is classified as nonhazardous, we strongly recommend using prudent laboratory practices: Avoid unnecessary contact, use of gloves, eye protection, lab coats, etc. when using this or any laboratory reagent.

Troubleshooting

<https://www.akoyabio.com/support/> | support@akoyabio.com

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