

# Opal Anti-Ms + Rb HRP Kit

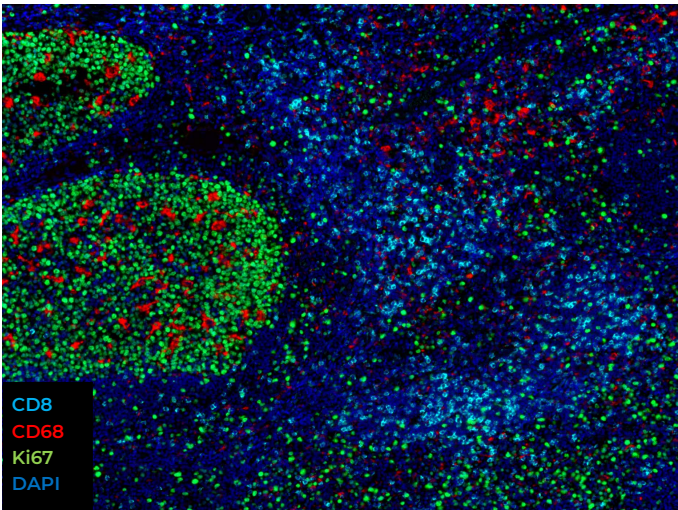
CATALOG # ARH3001KT

Opal Anti-Ms + Rb HRP Kit contains a concentrated secondary antibody and a diluent optimized for use with Opal® fluorescent dyes. Opal detection reagents provide exceptional signal amplification and low background for sub-cellular sensitivity staining. Use Opal Anti-Ms + Rb HRP secondary antibody on tissue treated with primary antibody raised in mouse or rabbit. This kit can be utilized for applications ranging from routine detection of co-localized biomarkers to detection of rare or low abundance proteins. Quantitative results for spatial biology analyses may be obtained using a Phenolmager® imaging system.

Components	
ARH3001EA	Opal Anti-Ms + Rb HRP, 1 tube (15 mL)
ARD3001EA	Opal Anti-Ms + Rb Diluent, 1 tube (40 mL)

Quantity
Up to 50 Slides (depending on automated or manual staining methods)

Storage and Stability			
Opal Reagent	Storage Temp	Shipping Temp	Stability
Opal Anti-Ms + Rb HRP	4°C	4°C	Stable until expiration date shown on product label when stored as directed.
Opal Anti-Ms + Rb Diluent	4°C	4°C	Stable until expiration date shown on product label when stored as directed.
Working Solution	4°C	N/A	Prepare fresh before each use. Discard unused working solution.



**FIGURE 1. Human FFPE tonsil tissue stained with Opal signal amplification system.** Multiplexed immunohistochemistry (mIHC) was performed using anti-CD68 (PGM1, 1:100 dilution) detected with Opal 620, anti-CD8 (4B11, 1:200 dilution) detected with Opal 480, anti-Ki67 (MIB1, 1:100 dilution) detected with Opal 570, and DAPI. Primary antibodies were diluted with 1X Opal Antibody Diluent/Block). Opal Anti-Ms + Rb HRP secondary antibody was diluted 4-fold and applied to the tissue for 10 minutes prior to Opal dye deposition. Antigen retrieval was performed at 97°C at pH 6. Slides were imaged at 20X magnification using the Phenolmager HT system.

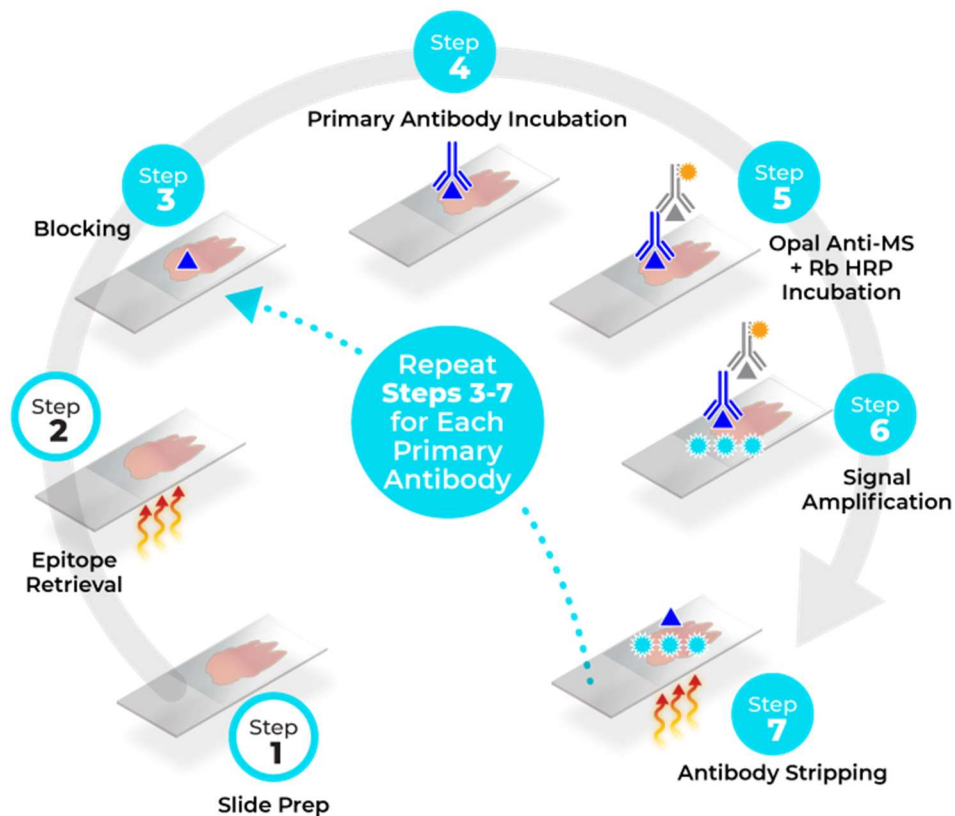
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### INSTRUCTIONS

#### Staining Protocol Guidelines

This kit is compatible with both manual and automated staining. An overview of the Opal assay protocol is shown for critical assay components in Figure 2 and is applicable to both manual and automated staining. Refer to the Opal Assay Development Guide for more detailed instructions.



**FIGURE 2. Overview of Opal Staining Protocol using Opal Anti-Ms + Rb HRP Kit.** Perform multiplexed staining using Opal Anti-Ms + Rb HRP Kit according to the steps in the workflow diagram. Bake FFPE slides at 65°C for 3 hours to remove paraffin (Step 1). Perform epitope retrieval at pH 6 or pH 9 at 97°C for 20 minutes. If staining manually, use AR6 Buffer (Catalog # AR6001KT) or AR9 Buffer (Catalog # AR9001KT) (Step 2). Block tissue using 1X Antibody Diluent/Block (Catalog # ARD1001EA), then incubate the first primary antibody for 30 minutes. Dilute primary antibody in 1X Antibody Diluent/Block. Antibody dilution can be determined empirically or by following manufacturer's recommendations (Steps 3 & 4). Dispense Opal Anti-Ms + Rb HRP secondary antibody and incubate for 10 minutes (Step 5). Deposit Opal dye to amplify signal and incubate for 10 minutes (Step 6). Heat strip antibody from tissue at 97°C for 20 minutes (Step 7). Repeat Steps 3-7 based on desired plex size.

**NOTE** An example of an automated Opal assay protocol stained on the Leica® BOND RX™ is provided at the end of this document and includes step-by-step instructions. One cycle is represented; repeat as needed based on desired plex size.

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### Working Solution Preparation

Opal Anti-Ms + Rb HRP Kit includes 2 components: Opal Anti-Ms + Rb HRP concentrated secondary antibody (ARH3001EA) and Opal Anti-Ms + Rb HRP Diluent (ARD3001EA). Create a working solution by diluting the concentrated secondary antibody with the provided diluent.

1. Retrieve Opal Anti-Ms + Rb HRP and Opal Anti-Ms + Rb Diluent from 4°C storage.
2. Prepare Opal Anti-Ms + Rb working solution by diluting Opal Anti-Ms + Rb HRP 4-fold in Opal Anti-Ms + Rb Diluent. If using an autostainer, account for dead volume based on the instrument. See Table 1 if preparing a 6-plex Opal assay run on the Leica BOND RX autostainer.

TABLE 1. Opal Anti-Ms + Rb HRP Reagent Volumes for Opal 6-plex Assay Stained on Leica BOND RX

Reagent	Volume for 6 Slides	Volume for 30 Slides
Opal Anti-Ms +Rb HRP	1.5 mL	7.5 mL
Opal Anti-Ms + Rb Diluent	4.5 mL	22.5 mL
Opal Anti-Ms + Rb Working Solution	6 mL	30 mL
Dead volumes have been accounted for and determined to be 300 µL for the 6 mL BOND Container and 1.6 mL for the 30 mL BOND Container. Each slide requires 150 µL/antibody stained (or 900 µL for a 6-plex).		

3. Vortex solution briefly to ensure a homogeneous mixture.

**NOTE** Refer to the *Opal Assay Development Guide* for detailed Opal assay protocol instructions. Technical Data Sheets for each Opal dye are available at [akoyabio.com](http://akoyabio.com).

## Opal Anti-Ms + Rb HRP Kit

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### FREQUENTLY ASKED QUESTIONS

1. Does this secondary antibody have higher reactivity with IgG or IgM primary antibodies?

This product reacts with IgM but has a higher affinity to IgG antibodies.

2. Is Opal Ms + Rb HRP secondary antibody cross-reactive with antibodies other than mouse and rabbit? For example, can this secondary antibody react with rat primary antibodies?

No, this product is unlikely to be cross-reactive with antibodies from other species.

3. Could dilution or incubation times be adjusted to reduce non-specific staining during optimization?

A dilution factor of 1:4 using the provided diluent and an incubation time of 10 minutes is recommended. However, dilution and incubation times can be adjusted as part of the staining optimization process.

4. Could a biotinylated secondary antibody be used with Opal assays or is an HRP-conjugated secondary antibody necessary?

High sensitivity detection using Opal dyes is achieved through tyramide signal amplification (TSA™) with HRP.

5. Could Opal Ms + Rb HRP secondary antibody be diluted with an alternative diluent such as PBS or TBS?

No, we do not support diluting the Opal Ms + Rb HRP secondary antibody with an alternative diluent.

6. Could the concentrated secondary antibody be used without the diluent?

We do not support or recommend using the concentrated secondary antibody without using the provided diluent.

7. What color is each reagent in the kit and what color is the working solution?

The concentrated secondary antibody stock solution is yellow and the diluent stock solution is clear. When combining concentrated secondary antibody and diluent to make a working solution, mix until the solution is homogeneous and light-yellow in color.

## Opal Anti-Ms + Rb HRP Kit

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### RECOMMENDED PRODUCTS

The following products are recommended for use with the Opal Anti-Ms + Rb HRP secondary antibody.

Product	Akoya Catalog #
1X Opal Antibody Diluent/Block	ARD1001EA
10X AR6 Buffer	AR6001KT
10X AR9 Buffer	AR9001KT
1X Plus Automation Amplification Diluent	FP1609
1X Plus Manual Amplification Diluent	FP1498
NEL871001KT	Opal 6-Plex Detection Kit - for Whole Slide Imaging
NEL861001KT	Opal 6-Plex Manual Detection Kit - for Whole Slide Imaging
Opal 480	FP1500001KT
Opal 520	FP1487001KT
Opal 570	FP1488001KT
Opal 620	FP1495001KT
Opal 690	FP1497001KT
Opal 780	FP1501001KT
10X Spectral DAPI	FP1490

## Opal Anti-Ms + Rb HRP Kit

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### AUTOMATED STAINING PROTOCOL - EXAMPLE

An example of an automated Opal staining protocol is provided using Opal Anti-Ms + Rb HRP secondary antibody and stained on the Leica BOND RX. One cycle of a multiplex assay is represented; repeat steps 4-26 as needed based on desired plex size.

**NOTE:** Dispense Opal reagents twice, including TSA-DIG. Opal 780 is dispensed last and requires a single dispense.

BOND RX				
Step Reagent	Supplier: N/A			
1. 1X PBS Buffer	Step type: Reagent	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
2. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
3. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya Biosciences			
4. 1X Antibody Diluent/Block	Step type: Reagent	Inc. (min): 5:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: N/A			
5. Primary Antibody	Step type: Reagent	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: N/A			
6. Primary Antibody	Step type: Reagent	Inc. (min): 30:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
7. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
8. Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
9. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya Biosciences			
10. Opal Anti-Ms + Rb HRP	Step type: Reagent	Inc. (min): 10:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
11. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
12. Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
13. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: Open
Step Reagent	Supplier: Leica Microsystems			
14. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya Biosciences			

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15. Opal Reagent	Step type: Reagent	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Akoya Biosciences			
16. Opal Reagent	Step type: Reagent	Inc. (min): 10:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
17. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
18. Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
19. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
20. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
21. Bond ER Solution (1 or 2)	Step type: Reagent	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
22. Bond ER Solution (1 or 2)	Step type: Reagent	Inc. (min): 0:00	Temp: 97°C	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
23. Bond ER Solution (1 or 2)	Step type: Reagent	Inc. (min): 20:00	Temp: 97°C	Dispense type: Intermediate
Step Reagent	Supplier: Leica Microsystems			
24. Bond ER Solution (1 or 2)	Step type: Reagent	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
25. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
26. Bond Wash Solution	Step type: Wash	Inc. (min): 1:00	Temp: Ambient	Dispense type: 150 µL
Step Reagent	Supplier: Leica Microsystems			
27. Bond Wash Solution	Step type: Wash	Inc. (min): 0:00	Temp: Ambient	Dispense type: 150 µL
Repeat steps 4-26 as needed based on desired plex size				

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