

# Primary Antibodies and Opal Pairings for Multiplex IHC

## TABLE OF PRIMARY ANTIBODY CLONES, INCLUDING VENDOR, DILUTION FACTOR, ANTIGEN RETRIEVAL BUFFER, AND OPAL FLUOROPHORE PAIRING.

Opal™ is a method for multiplex fluorescent immunohistochemistry in formalin-fixed, paraffin-embedded (FFPE) tissue. It allows the use of standard unlabeled primary antibodies, including multiple antibodies raised in the same species. The method involves detection with Opal reactive fluorophores that

covalently label the epitope. After labeling is complete, antibodies are removed in a manner that does not disrupt the Opal fluorescence signal. This allows the next target to be detected without fear of antibody cross-reactivity. Opal enables the development of multiplexed assays with balanced, quantitative signal for rare and abundant targets.

Marker	Clone	Vendor	DF	AR	Opal Pairing
CD3	SP7	Spring Biosciences	1:150	AR6, AR9	Opal 620, Opal 540
CD33	PWS44	Biocare Medical	RTU	AR6, AR9	Opal 780, Opal 570
CD4	4B12	Leica	RTU	AR9	Opal 540, Opal 620
CD8	C8/144B	Dako	1:200	AR6, AR9	Opal 480, Opal 520, Opal 570
CD20	L26	Biocare Medical	RTU	AR6, AR9	All Opals
CD68	PG-M1	Agilent	1:100, 1:200	AR6, AR9	Opal 620, Opal 520
CD163	MRQ-26	Cell Marque	1:100	AR6	Opal 620, Opal 570
FoxP3	236A/E7	Abcam	1:100, 1:150	AR6	Opal 570
Ki67	MIB1	Agilent	1:100, 1:300	AR6	Opal 570, Opal 650
CK	AE1/AE3	Biocare Medical	RTU, 1:300, 1:400	AR6	Opal 690, Opal 780
PD-1	EPR4877(2)	Abcam	1:200, 1:300	AR6	Opal 780
PD-L1	E1L3N	Cell Signaling Technologies	1:500	AR6, AR9	Opal 520
ATPase	EP1845Y	Abcam	1:100	AR6	Opal 780
CD45LcA	2B11+PD7/26	Dako	1:200, 1:250	AR6	Opal 690
CD45RO	T200/797	Abcam	1:100	AR6	Opal 570

Any IHC validated primary antibody will work with Opal, and in some cases, primary antibodies that are validated for other uses (Western blots) will work, as well. You are free to use the best antibody available for your target of interest. Additionally, optimized 7-color and 4-color IHC Immunology panel kits with Opal-antibody pairings are available. Visit <https://www.akoyabio.com/phenoptics/> for additional information.

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