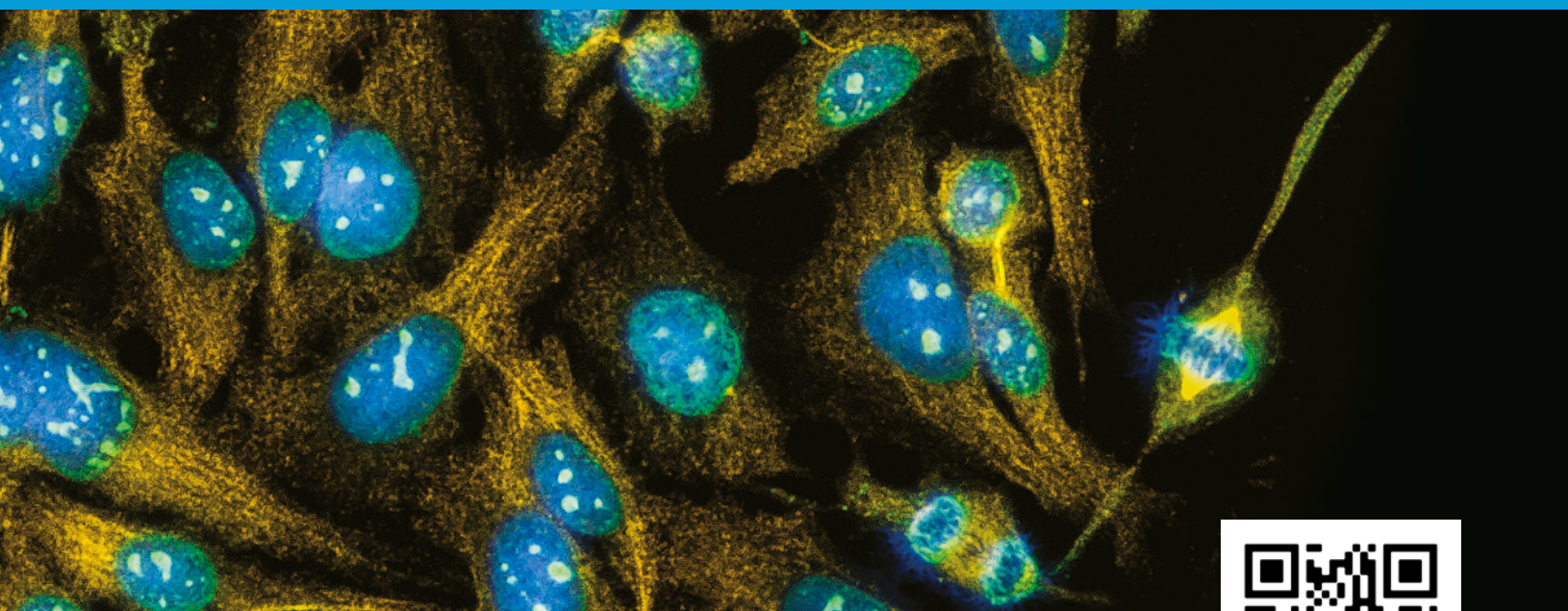


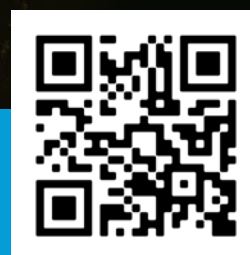
CONJUGATES DESIGNED FOR MULTIPLE LABELING

Four-Color Immunofluorescence



NEW Alexa Fluor® 555 and 568 Secondary Antibodies

Flexible Panel Design



Scan the code to access more information online

For immunofluorescence experiments with multiple targets, it's essential to use secondary antibodies cross-adsorbed against other species in the assay, which are conjugated to spectrally distinct fluorescent dyes. Here, we demonstrate how to combine **New Alexa Fluor® 555 and 568** conjugated secondary antibodies into your four-color immunofluorescence experiments.

	Fluorescent dyes	Excitation Peak	Emission Peak
	Alexa Fluor® 488	493 nm	519 nm
NEW	Alexa Fluor® 555	552 nm	572 nm
NEW	Alexa Fluor® 568	577 nm	602 nm
	Alexa Fluor® 594	591 nm	614 nm
	Alexa Fluor® 647	651 nm	667 nm
	Alexa Fluor® 680	684 nm	702 nm
	Alexa Fluor® 790	792 nm	803 nm

Four-Color Immunofluorescence

Four-Color Immunofluorescence Using DAPI, Alexa Fluor® 488, 555 and 647

The spectral characteristics of the three Alexa Fluor® dyes in this panel (**Figure 1**) allow for their combination with DAPI nuclear stain for effective four-color immunofluorescence. We demonstrated this color combination by staining Human epithelial (HEp-2) cells for common cell protein markers. Ki-67 protein, a marker of cellular proliferation (Scholzen and Gerdes., 2000), can be seen in green. Aspects of the cytoskeleton structure can be seen in yellow and were visualized by staining microtubules using anti- α tubulin to detect the heterodimeric filaments. cis-Golgi matrix protein, involved in the stacking of Golgi cisternae and maintenance of Golgi structure (Chang *et al.*, 2012), was visualized using Anti Human GM130/GOLGA2 and can be seen in red at the edge of the nucleus. The nucleus is stained blue using DAPI (**Figure 2**).

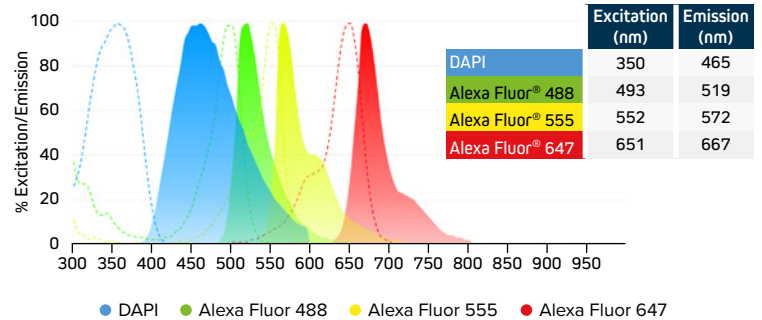


Figure 1

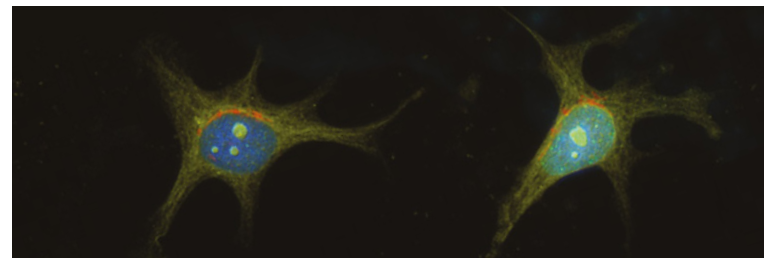


Figure 2: Indirect four-color immunostaining of Human epithelial (HEp-2) cells.

Four-Color Immunofluorescence Using DAPI, Alexa Fluor® 488, 568, and 647

Another useful combination of dyes that can be used with DAPI for four-color immunofluorescence is Alexa Fluor® 488, 568, and 647 (**Figure 3**). **Figure 4** demonstrates this color combination in Human epithelial (HEp-2) cells stained for common cell protein markers. Ki-67 protein, a marker of cellular proliferation (Scholzen and Gerdes., 2000), can be seen in green. Aspects of the cytoskeleton structure can be seen in yellow and were visualized by staining microtubules using anti- α tubulin to detect the heterodimeric filaments. Cell-to-cell contact was visualized by staining for the epithelial cell marker, E-Cadherin protein, which can be seen in red as filaments alongside the yellow microtubules. The nucleus was stained with DAPI and can be seen in blue.

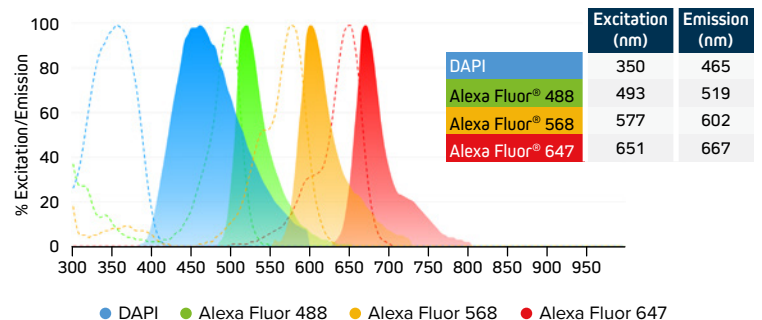


Figure 3

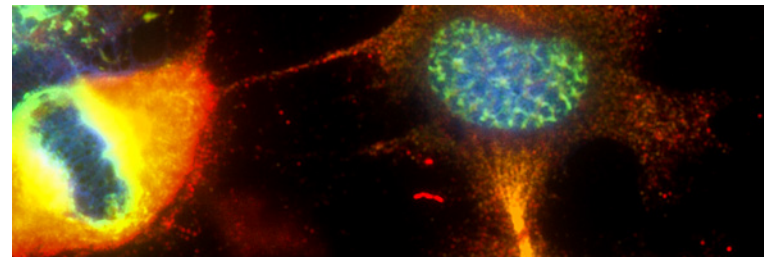


Figure 4: Indirect four-color immunostaining of Human epithelial (HEp-2) cells.

References:

Chang, S. H., Hong, S. H., Jiang, H. L., Minai-Tehrani, A., Yu, K. N., Lee, J. H., Kim, J. E., Shin, J. Y., Kang, B., Park, S., Han, K., Chae, C., & Cho, M. H. (2012). GOLGA2/GM130, cis-Golgi matrix protein, is a novel target of anticancer gene therapy. *Molecular therapy : the journal of the American Society of Gene Therapy*, 20(11), 2052-2063. <https://doi.org/10.1038/mt.2012.125>

Scholzen, T., & Gerdes, J. (2000). The Ki-67 protein: from the known and the unknown *Journal of cellular physiology*, 182(3), 311-322.

[https://doi.org/10.1002/\(SICI\)1097-4652\(200003\)182:3<311::AID-JCP1>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9)



Want more information?

Scan the code to access more information online!