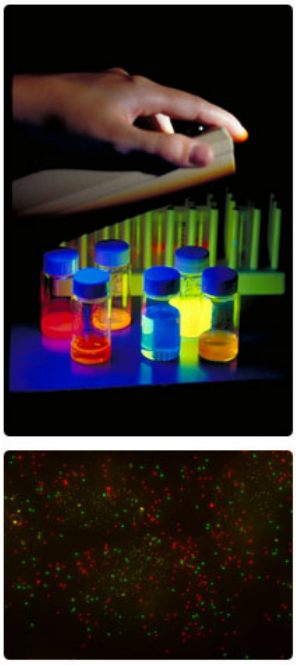
Stellaris® Dyes and Modifications

Here, you will find everything you need to know to choose the optimal fluorescent dye or other modification for your Stellaris® RNA FISH probe set(s). The most critical step to choosing a dye is checking compatibility between your filter set(s) and the dye that you wish to use. Even the best dye is useless for RNA FISH if you can't see it with your current microscope setup. Visit Chroma's spectra-viewer to view plots of our dyes in any combination to help plan multiplexing experiments. Other filter manufacturers besides Chroma may be compatible with our fluorophores. Please refer to your filter's specification sheet for compatibility.

	Modification	Alternative Dyes	Ex (nm)/ Em (nm)	Percentage of all Publications Click to visit publications
Fluorophores	FAM (Fluorescein)*	FITC	495/520	5%
	CAL Fluor® Orange 560	mKO/mOrange	538/559	<1%
	Quasar® 570	Cy3	548/566	25%
	TAMRA	TRITC	557/583	7%
	CAL Fluor Red 590	TAMRA	569/591	7%
	CAL Fluor Red 610	mCherry/TEXAS RED	590/610	10%
	CAL Fluor Red 635		618/637	<1%
	Quasar 670	Cy5	647/670	27%
	Biotin		n/a	0%



Human A549 cells probed with TOP1 with Quasar 670 (red), TFRC with Quasar 570 (green), and TERC with CAL Fluor Red 610 (yellow)

* Please note that due to variable auto-fluorescence in the green channel inherent to cells and tissue, we do not recommend use of the FAM for some Stellaris RNA FISH applications. We recommend testing your sample for green channel autofluorescence before ordering FAM labeled sets or ordering an alternative dye that is compatible with your experiment.

Fluorophores Manufactured By LGC Biosearch

MORE INFO

FAM (Fluorescein)	CAL Fluor Red 590
CAL Fluor Orange 560	CAL Fluor Red 610
Quasar 570	CAL Fluor Red 635
TAMRA	Quasar 670

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Biotin

Multiplexing Recommendations

Several Stellaris RNA FISH probe sets labeled with different dyes can be combined in the same experiment to provide insight into the localization of the selected RNA in relation to other RNAs and proteins. It is important to know which dyes are compatible for multiplexing in addition to the DAPI nuclear stain. Be sure to also take into account any other fluorophores used in protein immunofluorescence. Below are some multiplexing recommendations to help guide you in picking the right dyes for your experiment.

Relative brightness of a dye is highly dependent on having the proper equipment. When given the option between two similar dyes, pick the one that fits your filter, optics, and light source the best. Use Chroma's spectra-viewer and select your set or parts in the "Sets" or "Parts" tab.

Dye One (pick one): Quasar 570 or CAL Fluor Orange 560 dyes	**Dye Two**: Quasar 670 dye	**Dye Three** (pick one): CAL Fluor Red 610 or CAL Fluor Red 635 dyes	**Dye Four**: FAM
The Quasar 570 dye is bright, relatively photostable, and spectrally distinct from most other dyes. Most microscopes are already equipped to detect Quasar 570. The CAL Fluor Orange 560 dye can be picked if your equipment is better suited for this dye.	The Quasar 670 dye is our usual second choice due to its spectral distance from Quasar 570, which minimizes bleed-through in all but rare circumstances. The combination of Quasar 570 and Quasar 670 dyes is by far the most commonly published duplex RNA FISH dye pair and has been well vetted for success.	Both dyes are in between Quasar 570 and Quasar 670 but CAL Fluor Red 610 is a good third choice due to its brightness and compatibility with common filter sets. Bleed-through *may* be an issue between this dye and the two previous dyes depending on filters used. The CAL Fluor Red 635 dye is an alternative if your equipment is not optimized to detect CAL Fluor Red 610.	High auto-fluorescence is common to blue and green channels in both cells and tissues. Dyes that emit in the green part of the spectrum, like FAM, may only be useful under certain circumstances. If your experiment requires a fourth channel, you have a particularly strong probe set (35 probes or greater), and the RNA is distinct from other cellular features, FAM may be suitable for this purpose.

Please refer to Orjalo and Johansson. Meth Mol Bio. 2016 for an example of a successful quadruplex experiment.

For multiplexing with more than four dyes, please contact us at info@fisherbiotec.com for assistance.