REZOLVE SCIENTIFIC

FLUOROPHORES FOR TARGETED INSIGHTS

Tips and Tricks

Recommendations for best results with ReZolve products:

Step	Explanation	Recommendation
Fluorophore concentration/ dilution	There is a tendency to think more is better, but ReZolve products have an ideal concentration range. Using the dyes at too high a concentration can cause the dyes to precipitate and then they will not enter the cells.	All ReZolve dyes should be used at a final concentration of less than 50µM, and for most 10-20µM is recommended. Verify the protocol and recommended concentration for each product.
Sample preparation	All ReZolve dyes work well in live cells. However, there are some limitations in fixed cells preparation. For example: harsh fixation can remove lipids which many of our dyes require for localisation, sample permeablisation removes lipids, and paraffin embedded samples are generally not compatible with our dyes.	ReZolve recommend 2-4% PFA fixation for 10-20 minutes, to retain lipids which enable localisation.
Incubation time	ReZolve protocols are guidelines, and incubation times may need to be adjusted depending on the sample.	Longer or shorter incubation times may work better for your particular sample.
Mounting media or sample storage	Glycerol is a suitable mounting media, but can dull the signal and extract the dye if the sample is stored for too long before imaging.	Water based mounting medias are recommended with ReZolve dyes. For best results, image the sample the same day as staining.
Imaging	ReZolve protocols provide a general recommendation for imaging. However, each microscopy system is different and samples can vary greatly, and in somes cases emission / detection levels may seem faint.	Imaging can be improved by using lower capture speeds, or by leaving the detection open for longer to improve signal collection. The capture speed may be difficult to adjust when working with live samples, which can move during image collection.

REZOLVE SCIENTIFIC Pty Ltd
Office: Level 4, 12 Pirie Street, ADELAIDE SA 5000
Lab: University of SA Cancer Research Institute
North Terrace ADELAIDE SA 5000
Email: support@rezolvescientific.com Mobile: +61 (0) 409 195 323

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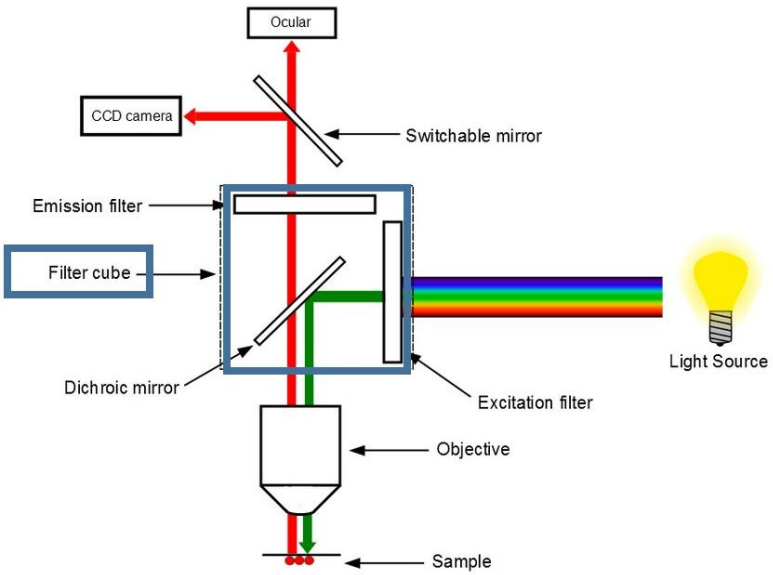
Imaging settings	ReZolve dyes excite with a UV or 405nm light source, unlike most blue excited dyes which have emission of 550-570 or 570-600. Some older system require filters for the excitation source, as well as the emitted light. If the filters are incorrect in epifluorescence microscopy, the excitation light can be blocked all together (see below for further explanation).	Verify the emission filter is set appropriately for imaging FITC or TexasRed dyes. Verify filters (excitation source and emission light filters) are correct when using older system. Confocal microscopy does not have this issue

Filter cubes in epifluorescence microscopy

The filter cube is an all-in-one unit of the microscope, which gets changed to allow the imaging of different fluorophores. The Excitation and Emission filters are a matched pair, based on organic fluorophores. Most older systems have 3 standard filter sets:

1. DAPI allow excitation by uv-405nm emission of ~420-470nm,
2. FITC – allows excitation by 488nm emission of ~500-550 nm,
3. TexasRed – allow excitation by 560nm emission of ~570-620nm).

The optimal filter cube for ReZolve dyes has a uv-450nm excitation filter and >420nm long band pass emission filter.



Please email support@rezolvescientific.com for further technical assistance.

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