

INTRODUCTION

Collagens and elastins are primarily synthesized by fibroblasts. These molecules are principal components of extracellular matrices. Once outside the cell, collagen assembles into fibrils and fibers that provide mechanical strength to tissues. Elastin is secreted by cells, and also forms fibers which crosslink to create a flexible network of fibers and sheets.

Col-F is a low molecular weight fluorescent probe that exhibits affinity for collagen and elastin. Col-F easily penetrates between cells and into tissues where it can then bind to collagen and elastin fibers via a noncovalent mechanism. Col-F is recommended for staining fresh or frozen tissues. Col-F is not recommended for use with fixed tissues.

Each vial contains 0.5 mg of dry powder Col-F. Once reconstituted in DMSO, Col-F is ready to use: just add it to the sample media, incubate, wash, and analyze. The optimal excitation is 490 nm and emission is 515-520 nm. Col-F is revealed under a microscope using a FITC-compatible filter set. Col-F is for research use only. Not for use in diagnostic procedures.

SPECIFICATIONS

- 0.5 mg per vial
- MW: 735.85 g/mol

STORAGE

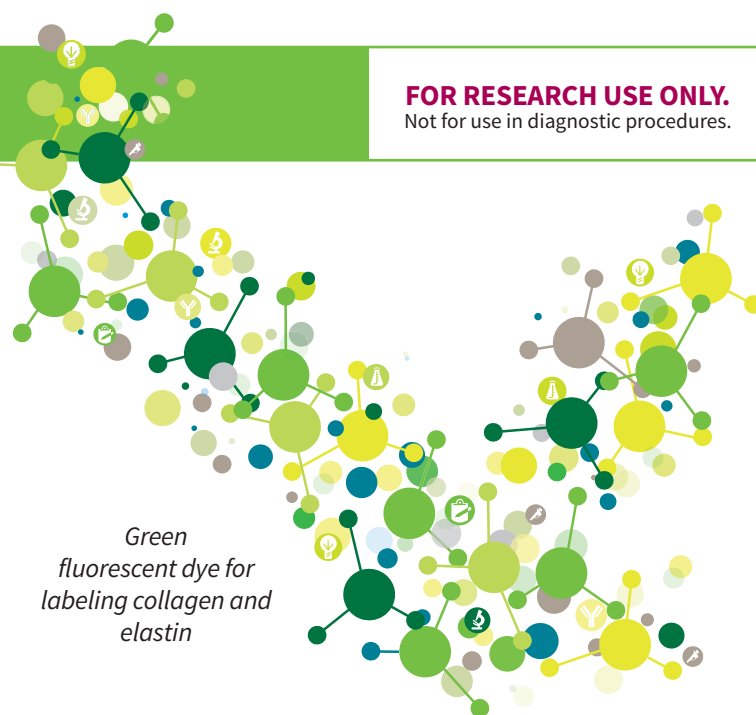
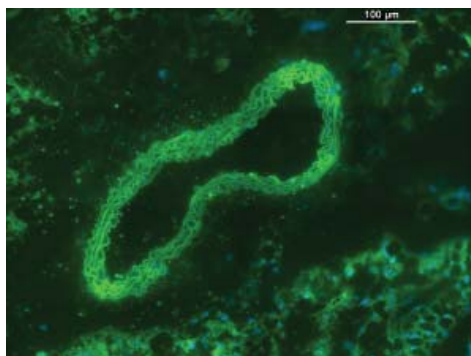
- Store $\leq -20^{\circ}\text{C}$
- Avoid freeze/thaw cycles.

SAFETY

- See Safety Data Sheet (SDS) for any warnings.
- SDS available at www.immunochemistry.com and by calling ICT.
- For research use only. Not for use in diagnostic procedures.

FIGURE 1: MICROSCOPY IMAGING

Fluorescence microscopy imaging of a frozen murine tissue section containing a blood vessel stained with 1 μM Col-F for 30 minutes. Collagen and elastin present in the blood vessel wall stained green. Nuclei were counter-stained blue with DAPI. Image was captured using a Nikon TiE Deconvolution Microscope. Data courtesy of Dr. Mike Olin, University of Minnesota.



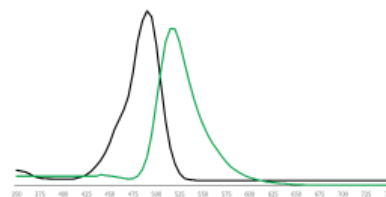
*Green
fluorescent dye for
labeling collagen and
elastin*

HOW TO USE

Col-F is supplied as a lyophilized powder. Typical staining concentrations range between 1-20 μM . However, the optimal staining concentration will vary for different situations and therefore needs to be determined experimentally by the end user. The ideal staining time varies depending on application, and can range from 5-10 minutes to several hours. Optimal staining times or periods will need to be determined experimentally by the user. ICT recommends staining for 30 minutes as a starting point. To stain collagen and elastin:

1. When ready to use, reconstitute the 0.5 mg Col-F vial by adding 100 μL DMSO (this creates a stock at 6.8 mM).
2. Optional: Incubate samples with blocking buffer for 60 minutes.
3. Add Col-F to the media/buffer for each appropriate sample.
4. Incubate the samples with the dye solutions for 5-60 minutes at 37°C .
5. Wash samples twice with PBS (allow samples to incubate at room temp for 5-10 minutes in PBS during each wash step).
6. Once wash steps are complete, mount a coverslip on each slide. Samples are now ready to visualize with a fluorescence microscope capable of excitation at 490 nm, and emission at 515-520 nm.

FIGURE 2: FLUORESCENCE SPECTRA



BRIGHT MINDS, BRIGHT SOLUTIONS.

ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.